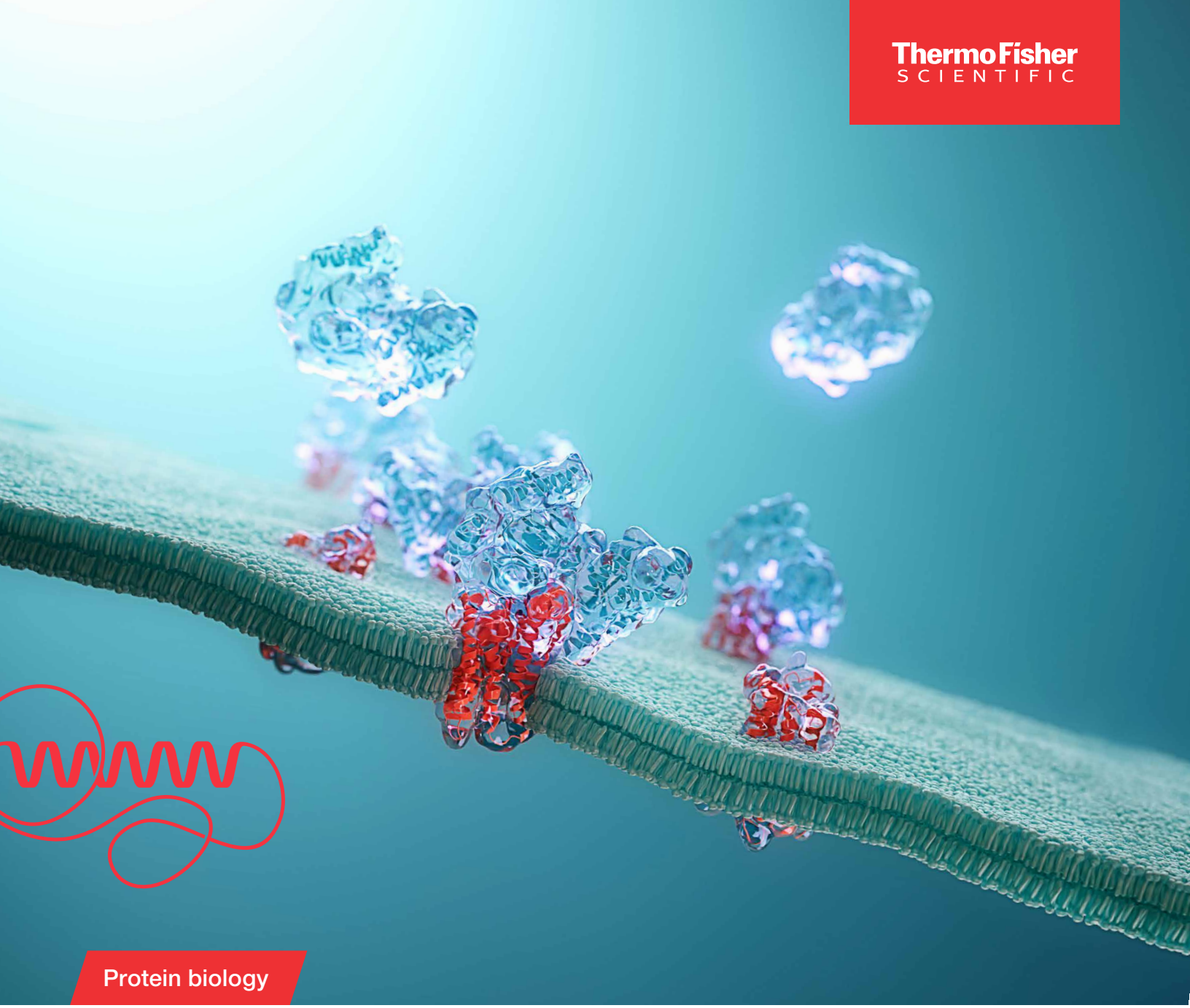


Protein biology

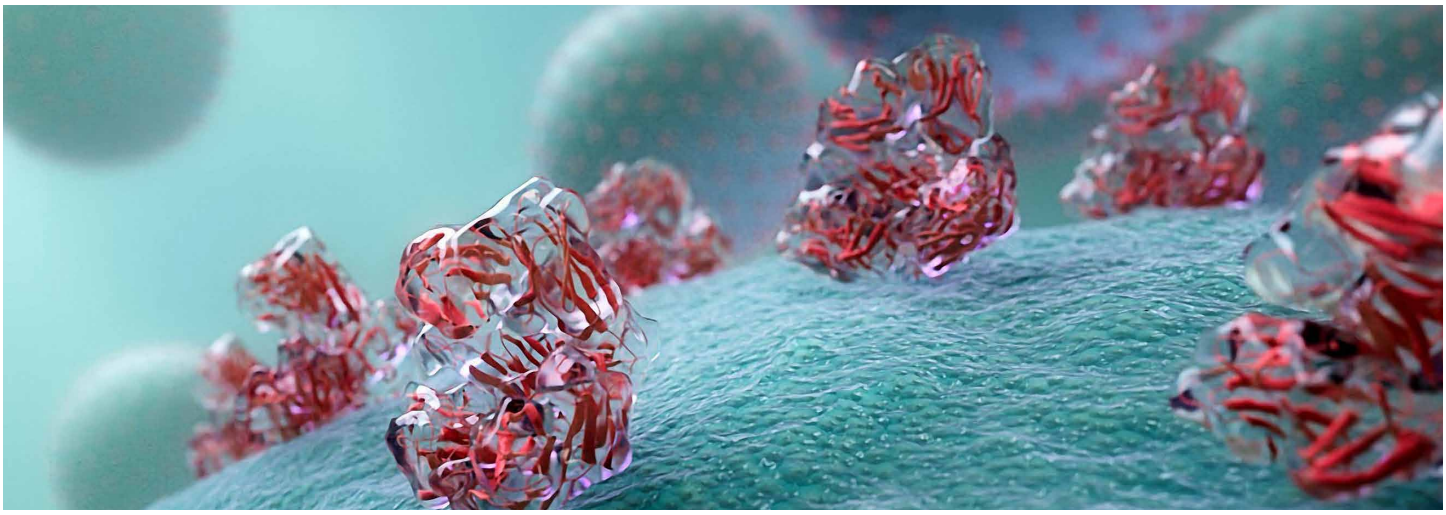
Protein research handbook

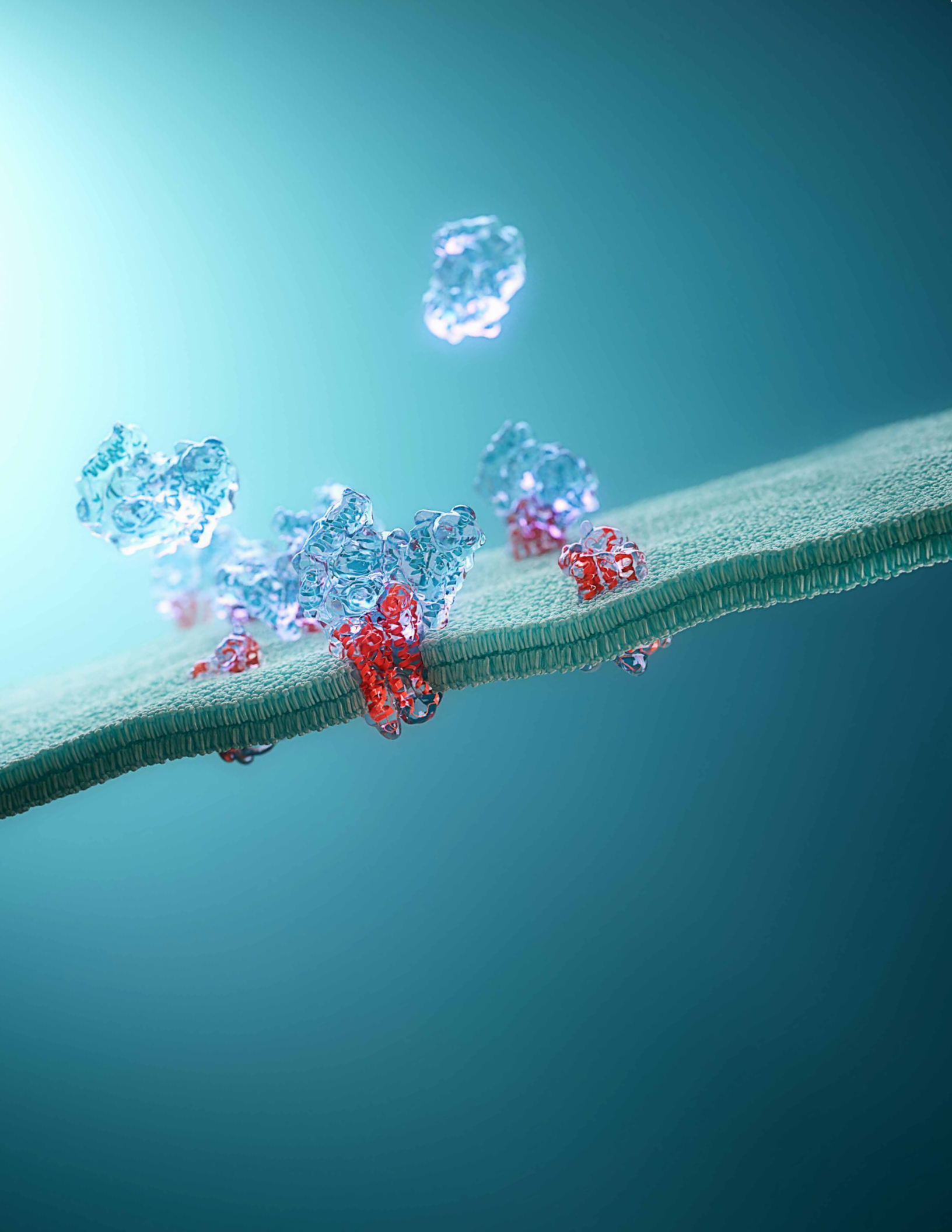
Cloning and protein expression • Protein sample preparation • Protein purification • Gel electrophoresis • Western blotting • Immunoassays • Mass spectrometry • Modification and crosslinking • Cryo-EM sample preparation

Detect, measure, and analyze protein expression, identity, and function using our portfolio of protein biology products.

Protein biology encompasses both the study of the structure and function of proteins as the primary focus of investigation and the use of antibodies, proteins, and peptides as tools to purify, detect, and characterize biological systems. Certain methods of protein analysis, such as immunoprecipitation, western blotting, and ELISA, have been used routinely in laboratories for many years. Others, such as quantitative protein mass spectrometry and cryo-EM, are relatively recent and rapidly developing technologies. New tools and products are continually being developed to advance all aspects of protein biology research.

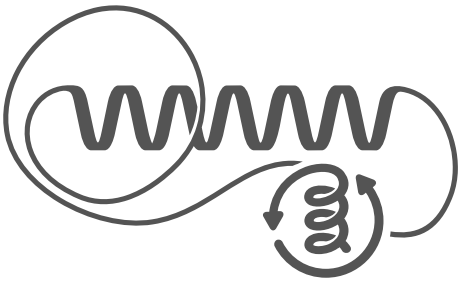
Contents

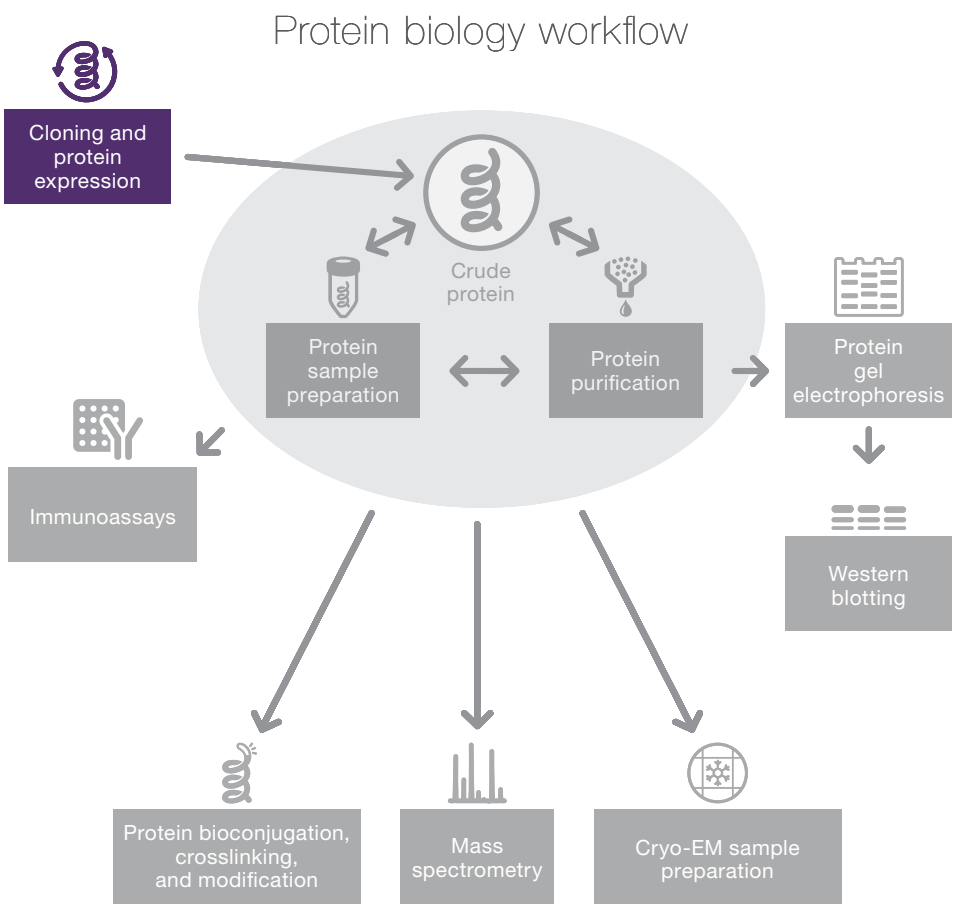




Cloning and protein expression

Recombinant protein expression technology enables analysis of gene regulation and protein structure and function. Utilization of recombinant protein expression varies widely—from investigation of function *in vivo* to large-scale production for structural studies and biotherapeutic drug discovery.





Introduction

To investigate how particular proteins regulate biology, researchers usually require a means of producing (manufacturing) functional proteins of interest. Given the size and complexity of proteins, *de novo* synthesis is not a viable option for this endeavor. Instead, living cells or their cellular machinery can be harnessed as factories to build and construct proteins based on supplied genetic templates (Figure 1).

Figure 1. Transcription and translation. Information is flowed from DNA base-pair sequence (gene) to amino acid polypeptide sequence (protein).

In prokaryotes, the processes of transcription and translation occur simultaneously. In eukaryotes, the processes are spatially separated and occur sequentially—with transcription happening in the nucleus and translation occurring in the cytoplasm. After translation, polypeptides are modified in various ways to complete their structure, designate their location, or regulate their activity within the cell. Posttranslational modifications (PTMs) are various additions or alterations to the chemical structure of the newly synthesized protein and are critical features of overall cell biology.

Cloning refers to the propagation of DNA of interest from an existing organism. This can be accomplished by cloning a gene of interest into an expression vector (Figure 2).

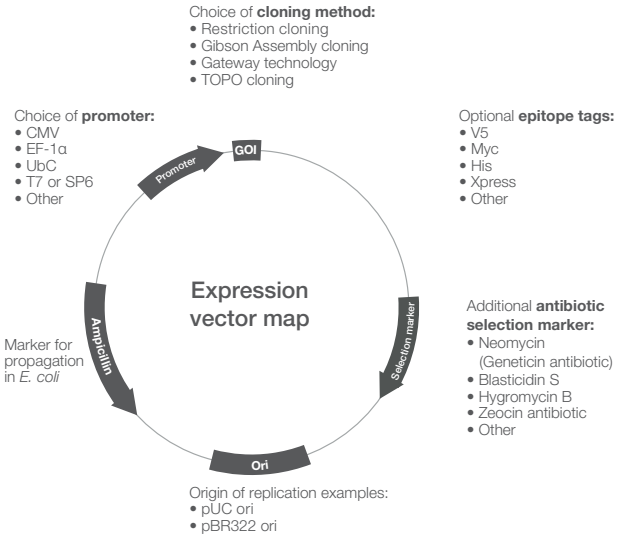


Figure 2. Example of an expression vector.

Most vectors contain a promoter for expression by a specific host system; however, some offer the option to add your own promoter. Once cloning is completed, plasmids are taken up into competent cells (e.g., chemically competent or electrocompetent *E. coli*) for propagation and storage, by a process called transformation. Epitope tags can be used to allow for easy detection or rapid purification of your protein of interest by fusing a sequence coding for the tag to your gene (Table 1).

Table 1. Overview of epitope tags.

Purpose	Description	Examples of tags
Detect	Well-characterized antibody available against the tag; easily visualized	V5, Xpress, Myc, 6xHis, GST, BioEase, capTEV, GFP, Lumio, HA, FLAG tag
Purify	Resins available to facilitate purification	6xHis, GST, BioEase, capTEV tag
Cleave	Protease recognition site (TEV, EK, HRV 3C, factor Xa) to remove tag after expression to obtain native protein	Any tag with a protease recognition site following the tag (only on N terminus)

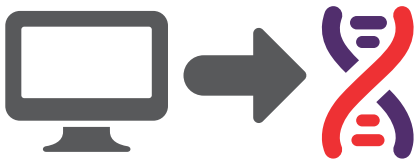
Using the right expression system for your specific application is the key to success. Protein solubility, functionality, purification speed, and yield are often crucial factors to consider when choosing an expression system. Additionally, each system has its own strengths and challenges, which are important when choosing an expression system. For example, bacterial host cells are low-cost, easy to culture, and easily scalable but are limited to the expression of bacterial proteins or simple eukaryotic proteins with limited posttranslational modifications. Since most proteins undergo some degree of posttranslational modification, bacterial host cells limit the range of the proteins expressed.

Insect and mammalian host systems support the most complex proteins and maximum protein quality. Insect host systems support multi-protein complexes with posttranslational modifications similar to mammalian cells and are a good option for proteins that are toxic to mammalian cells. If the protein studied must be identical to its *in vivo* mammalian counterpart, then a mammalian host system is ideal. Mammalian host systems retain the most posttranslational modifications and most closely resemble functional human proteins. However, these systems are also the most expensive. Both insect and mammalian cell lines have more demanding culture conditions, and therefore are not suited to every protein expression case. The most common mammalian cell lines are human embryonic kidney (HEK293) cells and Chinese hamster ovary (CHO) cells.

Choice of cell line may also depend on whether the protein of interest expresses better in one cell line or the other, or whether the researcher wants to take a transient or stable expression strategy. Transient expression usually implies a short-term, small-scale production, while stable expression involves the long-term integration of genes into the genome for large-scale production. However, new technologies have transformed transient expression to have robust yields.

Tools to optimize your cloning step

GeneArt Gene Synthesis and GeneOptimizer software



The power of custom gene synthesis is the ability to design your DNA without the constraints of traditional cloning. Equally important to most researchers, however, is obtaining high yields of mRNA and, ultimately, protein from synthetic genes. We developed **Invitrogen™ GeneArt™ GeneOptimizer™ software** to maximize the expression of synthetic genes in all commonly used expression systems.

Tuning the expression level by choosing the optimal promoter and terminator combination can be an essential part of your expression project. The origin of replication also has a significant influence on the expression level of the foreign protein. We offer a broad range of commercially available, predesigned vectors optimized for various expression systems.

Learn more at **thermofisher.com/genesynthesis**

Gibson Assembly kits and GeneArt Strings DNA Fragments

Invitrogen™ GeneArt™ Gibson Assembly® cloning kits provide highly efficient, seamless cloning, enabling the assembly of multiple DNA fragments of varying length into any vector.

When combined with **Invitrogen™ GeneArt™ Strings DNA Fragments** or **Invitrogen™ GeneArt™ Gene Synthesis**, these cloning kits can be used to build simple constructs as well as large and demanding constructs from multiple fragments.

Learn more about GeneArt Gibson Assembly cloning kits for protein expression at **thermofisher.com/gibsonassembly**

Gateway cloning

Invitrogen™ Gateway™ cloning technology is rapid, robust, and suited for high-throughput clone generation for protein production. It allows shuffling between different expression systems in just a few simple steps. Choose from a diverse selection of host systems, including *E. coli*, yeasts, and insect or mammalian cells, each of which utilizes unique destination vectors for all your expression applications. Additionally, the Invitrogen™ Gateway™ Vector Conversion System can convert specialized or customized vectors into an Invitrogen™ Gateway™ destination vector to suit your expression workflow.

Select the right Gateway vectors for your workflow and review our cloning protocols at **thermofisher.com/gateway**

One Shot chemically competent *E. coli*

E. coli cells are widely used for production of recombinant proteins quickly, economically, and on a large scale. The most popular strain for recombinant protein expression is BL21 and its derivatives. Invitrogen™ One Shot™ chemically competent cells, such as BL21 Star™ (DE3) and BL21-AI™ cells, are optimized for high-level protein or toxic protein expression from T7 promoters. All are induced with IPTG or L-arabinose and come in the convenient Invitrogen™ One Shot™ format, allowing for transformation and recovery in a single tube.

Choose chemically competent cells optimized for protein expression at **thermofisher.com/compcells**

Recombinant protein expression systems

Achieving optimal and reliable amounts of recombinant protein is easier because of our wide selection of trusted mammalian, insect, yeast, bacterial, and cell-free protein expression systems (Table 2). Backed by a team of experienced professionals to help you quickly optimize your protein expression experiments, we can accelerate your research and development by offering:

- Higher protein yields (3–20x higher than other systems)
- Faster protein production (days vs. weeks or months)
- High cell density
- Lower cost per mg of protein
- Complete, optimized systems of cells, media, transfection reagents, enhancers, feeds, and vectors

The Gibco™ Expi™ platforms are examples of expression systems that are fast and cost-effective with high yield. These systems combine the advantages of both transient and stable strategies for high expression of diverse proteins (Figure 3). For example, the Gibco™ Expi293™ Expression System uses HEK293 cells and can yield up to 1 g/L of protein in just 5–7 days.

Table 2. Expression system selection guide.

	Human (HEK293)	Hamster (CHO)
Expression system	**Human (HEK293)**	**Hamster (CHO)**
Featured application	Membrane proteins (GPCRs, ion channels, other membrane proteins)	Ig-related proteins
Other applications	• Difficult-to-express proteins • Secreted proteins	• Membrane proteins • Secreted proteins
Posttranslational modifications	Full	Nearly full
Protein yield	Up to 1 g/L	Up to 3 g/L
Recommended host system kit	Expi293 expression system kits	ExpiCHO Expression System Kit
Recommended cells	Expi293F Cells Expi293F GnTI⁻ Cells Expi293F Inducible Cells Expi293F Inducible GnTI⁻ Cells	ExpiCHO-S Cells (cGMP Banked) ExpiCHO-S Cells
Recommended media	Expi293 Expression Medium	ExpiCHO Expression Medium
Recommended transfection and delivery	ExpiFectamine 293 Transfection Kit	ExpiFectamine CHO Transfection Kit
Recommended expression vectors	pcDNA3.4 TOPO vector	pcDNA3.4 TOPO vector
Recommended cultureware	Nalgene shake flasks Nunc bioreactor tubes Nunc plates	Nalgene shake flasks Nunc bioreactor tubes Nunc plates
Recommended extraction reagent kit	M-PER Mammalian Protein Extraction Reagent	M-PER Mammalian Protein Extraction Reagent
Recommended protein labeling	Expi293 Met (–) Protein Labeling Kit L-Methionine (Methyl-^{13}C) L-Selenomethionine	
Scale-up	Expi293 Expression Medium Single-use bioreactor vessels	ExpiCHO Stable Production Medium EfficientFeed C+ 2X Supplement Efficient-Pro Feed 2 Single-use bioreactor vessels
Services option	Gene-to-protein services	Gene-to-protein services

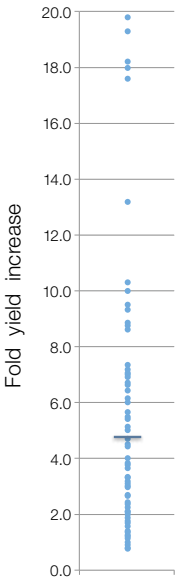


Figure 3. External collaborator results. In collaboration with 22 labs, expression levels of 98 different proteins were tested using the Expi293 Expression System. The following results were obtained:

- 87% of proteins demonstrated increased expression in the Expi293 system compared with the user's current system
- 4.6x average increase for all proteins (n = 98)
- 4.0x average increase for mAbs (n = 54); highest level was 826 mg/L
- 5.3x average increase for non-mAbs (n = 44); highest level was 790 mg/L

Insect (Sf9, Sf21)	Yeast	Bacterial	Cell-free
Intracellular proteins	Industrial enzymes	Bacterial proteins	Rapid protein expression
• Toxic proteins • Multi-protein complexes	Low-complexity proteins	Low-complexity proteins	• Toxic proteins • *In vitro* labeling
Partial	Partial	Limited	Partial
Up to 900 mg/L	Up to 10 g/L	Up to 10 mg/L	Up to 750 mg/L
ExpiSf Expression System Starter Kit	EasySelect *Pichia* Expression Kit	Champion pET SUMO Expression System pBAD TOPO TA Expression Kit	Cell-free *in vitro*
ExpiSf9 Cells	PichiaPink Expression Strain Set GS115, *Pichia pastoris* Yeast Strain X-33, *Pichia pastoris* Yeast Strain	One Shot BL21 Star (DE3) Chemically Competent *E. coli* cells BL21-AI One Shot Chemically Competent *E. coli* cells	HeLa and CHO extracts
ExpiSf CD Medium	PichiaPink Media Kit YPD Broth	MagicMedia *E. coli* Expression Medium LB Broth	None required
ExpiFectamine Sf Transfection Reagent	Neon Transfection System Starter Pack	None required	None required
pFastBac 1 vector pFastBac Dual Expression Vector	pPINK (LC and HC) pPICZα A, B, and C *Pichia* Vectors pPICZ A, B, and C *Pichia* Vectors	Champion pET SUMO vector pBAD TOPO vector pRSET bacterial expression vectors	T7 cell-free expression vectors
Nalgene shake flasks Nunc bioreactor tubes Nunc plates	Nalgene shake flasks	Nalgene shake flasks	
I-PER Insect Cell Protein Extraction Reagent	Y-PER Yeast Protein Extraction Reagent	B-PER Complete Bacterial Protein Extraction Reagent	None required
ExpiSf CD Medium Single-use bioreactor vessels		Bacto CD Supreme Fermentation Production Medium (FPM) Single-use bioreactor vessels	
Gene-to-protein services			

Gibco™ ExpiCHO™ Expression System

- Maintain protein quality and function and easily transition from research to production using the Gibco™ ExpiCHO-S™ cGMP-banked cell line and Gibco™ ExpiCHO™ Stable Production Medium
- Get the highest yields possible in a transient expression system (up to 3 g/L).

Learn more at **thermofisher.com/expicho**

Gibco™ Expi293™ Expression System Kit

- Rapid production of protein in just 5–7 days
- Specialized cell lines for structural biology research, such as Gibco™ Expi293F™ cell lines, are available

Learn more at **thermofisher.com/expi293**

Gibco™ ExpiSf™ Expression System

- First chemically defined baculovirus expression system
- Generate 3x more protein compared to existing insect expression platforms

Learn more at **thermofisher.com/expisf**

Test your protein research knowledge

Question: Which of the following are some of the most common mammalian cell lines used for foreign protein expression? (Select all that apply.)

A. HeLa

B. CHO

C. Jurkat

D. HEK293

Answer: B and D

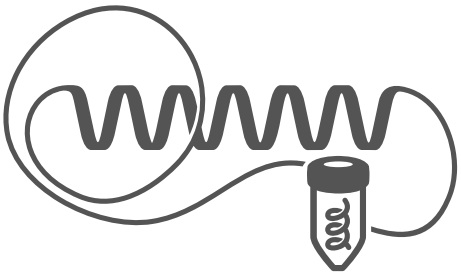
Ordering information

Product	Quantity	Cat. No.
Mammalian cell–based systems		
Expi293 Expression System Kit	1 kit	A14635
Expi293 Expression Medium	1,000 mL	A1435101
ExpiFectamine 293 Transfection Kit	1 kit	A14524
Expi293 GnTI– Expression System Kit	1 kit	A39250
ExpiCHO Expression System Kit	1 kit	A29133
ExpiCHO Expression Medium	1,000 mL	A2910001
ExpiFectamine CHO Transfection Kit	1 kit	A29129
To view additional products, go to **thermofisher.com/mammalianexpression**		
Insect cell–based systems		
ExpiSf Expression System Starter Kit	1 kit	A38841
ExpiSf CD Medium	500 mL	A3767801
ExpiFectamine Sf Transfection Reagent	1 mL	A38915
To view additional products, go to **thermofisher.com/insectexpression**		
Yeast cell–based systems		
PichiaPink Secretion Optimization Kit	1 kit	A11150
EasySelect *Pichia* Expression Kit	1 kit	K174001
Pichia Expression Kit, original kit	1 kit	K171001
To view additional products, go to **thermofisher.com/yeastexpression**		
Bacterial cell–based systems		
GeneArt Gibson Assembly HiFi Master Mix	50 reactions	A46628
Gateway LR Clonase II Enzyme Mix	20 reactions	11791020
Champion pET100 Directional TOPO Expression Kit with BL21 Star (DE3) One Shot Chemically Competent *E. coli*	20 reactions	K10001
pBAD202 Directional TOPO Expression Kit	20 reactions	K420201
One Shot BL21 Star (DE3) Chemically Competent *E. coli*	20 reactions	C601003
BL21-AI One Shot Chemically Competent *E. coli*	20 reactions	C607003
MagicMedia *E. coli* Expression Medium	1 L	K6803
To view additional products, go to **thermofisher.com/bacterialexpression**		
Cell-free systems		
1-Step Human Coupled IVT Kit – DNA	40 reactions (25 µL each)	88882
1-Step Human High-Yield Mini IVT Kit	10 reactions (100 µL each)	88891
1-Step Human High-Yield Maxi IVT Kit	2 reactions (2 mL each)	88892
Retic Lysate IVT Kit	60 reactions	AM1200
To view additional products, go to **thermofisher.com/cellfreeexpression**		

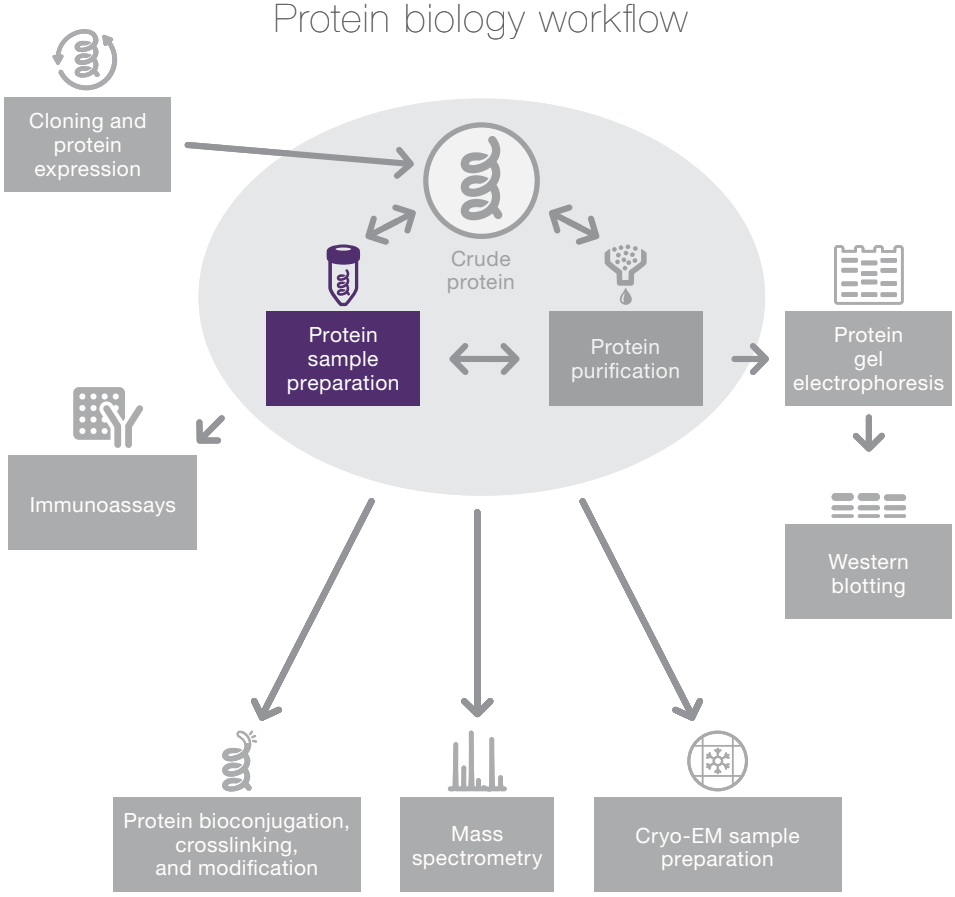
For more information, or to view additional pack sizes, expression vectors, media, and cell lines, go to **thermofisher.com/proteinexpression**

Protein sample preparation

Because of the heterogeneity of proteins, there is no one method or reagent that is optimal for general protein isolation and purification. The first step in protein analysis is cellular extraction. Following lysis, and depending on the next step in the workflow, the protein extract may require further cleanup, using techniques such as dialysis, desalting, or concentration. After any cleanup procedure, the amount of protein in the sample should be determined using any one of numerous protein assays.



Protein biology workflow



Protein extraction

Protein extraction techniques vary depending on the source of the starting material, the location within the cell of the protein of interest, and the downstream application. Historically, mechanical disruption has been used to lyse cells and tissues, but detergent-based solutions have more recently been developed to efficiently lyse cells and enable the separation of subcellular structures without requiring physical methods. However, cell lysis disrupts cell membranes and organelles resulting in unregulated proteolytic activity that can reduce protein yield and function. To prevent these negative effects, protease and phosphatase inhibitors can be added to the lysis reagents. Numerous compounds have been identified and used to inactivate or block the activities of proteases and phosphatases by reversibly or irreversibly binding to them.

Protein cleanup

Many detergents and salts used in protein extraction formulations may have adverse effects on protein function or stability, or may interfere with downstream analysis; therefore, it may be necessary to remove or reduce these contaminants following cell lysis, using techniques such as dialysis or desalting. In addition, if the protein sample is too dilute, it may require concentration.

Dialysis facilitates the removal of small unwanted compounds from protein in solution by selective diffusion through a semipermeable membrane. Proteins that are larger than the membrane pores are retained on the side of the membrane, but small contaminants diffuse freely through the membrane and approach an equilibrium concentration.

Desalting, also known as size exclusion chromatography or gel filtration, utilizes a resin with pores that are large enough for small contaminants (e.g., salts) to penetrate, but too small for the protein of interest to enter. This causes the contaminants to slow down their rate of migration, and the larger, faster proteins separate from the slower, smaller molecules.

Protein concentration and diafiltration use a semipermeable membrane to separate macromolecules from low molecular weight compounds via centrifugation. During concentration, the low molecular weight solutes are forced through the membrane while the macromolecules remain on the sample side of the membrane, where they become concentrated to a smaller volume as the reagent is forced across the membrane to the opposite side.

Protein assays

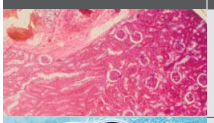
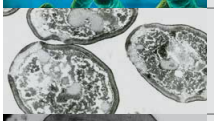
Depending on the accuracy required and the amount and purity of the protein available, different methods are appropriate for determining protein concentration. Colorimetric, reagent-based protein assay techniques have been developed that are used by nearly every laboratory involved in protein research. Protein is added to the reagent, producing a color change in proportion to the amount added. Protein concentration is determined by reference to a standard curve consisting of known concentrations of a purified reference protein. Unfortunately, no protein assay method exists that is either perfectly specific to proteins or uniformly sensitive to all protein types. Therefore, successful use of protein assays involves selecting the method that is most compatible with the samples to be analyzed, choosing an appropriate assay standard, and understanding and controlling the particular assumptions and limitations that remain.

Protein extraction reagents and kits

Gentle formulations designed to maximize protein yield and activity

Obtain high protein yield from tissues, cells, or subcellular fractions using reagents and kits that are optimized for mammalian, bacterial, yeast, insect (baculovirus), and plant samples (Table 1). These gentle formulations have been verified in multiple tissue types and cell lines, and generally eliminate the need for mechanical cell disruption. These extracts are compatible with a wide range of downstream applications, including protein assays, immunoprecipitation, protein purification, immunoassays, western blotting, gel shift assays, and enzyme assays.

Table 1. Overview of sample types and protein extraction reagents and kits.

	Sample type	Goal	Recommended Thermo Scientific™ reagents or kits	
	**Primary or cultured mammalian cells or tissues**	• Total protein extraction	• M-PER reagent • T-PER reagent • N-PER reagent	• RIPA Lysis and Extraction Buffer • Pierce IP Lysis Buffer
	**Cultured mammalian cells or tissues**	• Subcellular fractionation or organelle isolation	• NE-PER reagent • Subcellular Fractionation Kits • Mitochondria Isolation Kits	• Pierce Cell Surface Protein Isolation Kit • Syn-PER Reagent • Lysosome Enrichment Kit
	**Bacterial cells**	• Total protein extraction	• B-PER reagent	
	**Yeast cells**	• Total protein extraction	• Y-PER reagent	
	**Insect cells (baculovirus)**	• Total protein extraction	• I-PER reagent	
	**Plant tissue (leaf, stem, root, flower)**	• Total protein extraction	• P-PER reagent	

Highlights:

- **Optimized**—formulations maximize protein yield and preserve protein activity (Figure 1)
- **Efficient**—only produces minimal cross-contamination between subcellular fractions (Figure 2)
- **Compatible**—extracts can be used directly in most downstream applications
- **Gentle**—eliminates the need for mechanical cell disruption for most sample types

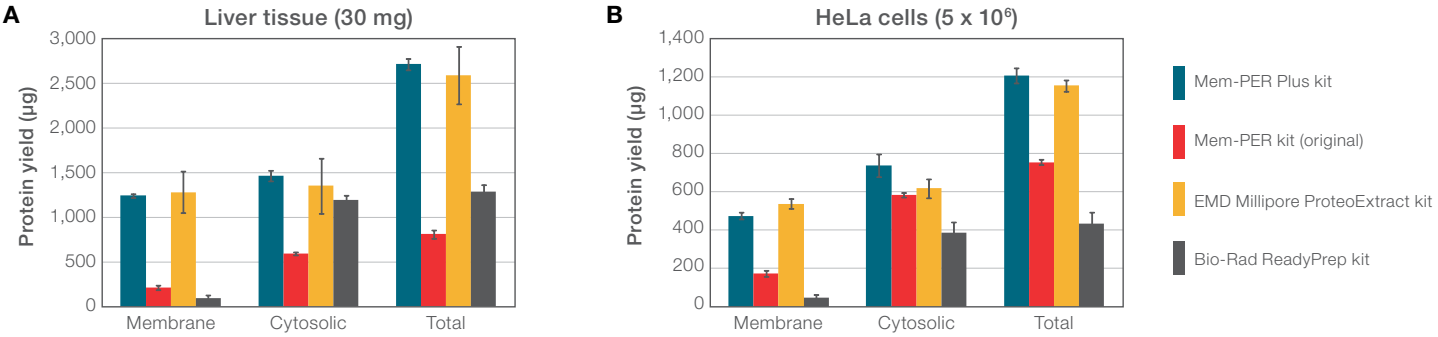


Figure 1. Improved protein yield using the Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit. Membrane proteins were isolated from mouse liver tissue and HeLa cells using four commercial extraction kits. Protein yields for membrane, cytosolic, and total fractions were determined using the Thermo Scientific™ Pierce™ BCA Protein Assay Kit.

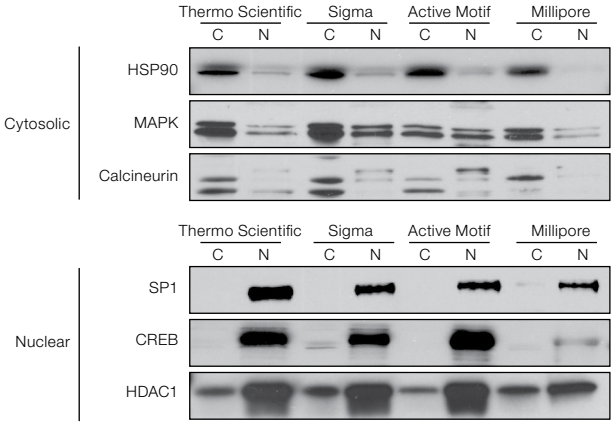


Figure 2. Nuclear and cytosolic fractions are obtained with minimal cross-contamination. HeLa cell proteins were extracted with Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Reagents or with nuclear extraction kits from other vendors. Samples of the nuclear and cytosolic fractions were analyzed by western blot using antibodies against common nuclear and cytoplasmic protein markers and visualized using Thermo Scientific™ SuperSignal™ West Pico Chemiluminescent Substrate. Nuclear fractions produced with the NE-PER kit had minimal to no contamination with cytosolic proteins.

For more information, or to view additional products, go to **thermofisher.com/proteinextraction**

Detergents

Easy-to-pipette, highly purified Surfact-Amps 10% solutions

Thermo Scientific™ Surfact-Amps™ Detergent Solutions are easy-to-use 10% (w/v) solutions of highly purified detergents that can be used in routine and high-demand protein research methods and molecular biology techniques (Figure 3). These formulations provide high purity, quality, and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% solutions are easy to pipet accurately. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules or non-leaching HDPE bottles, helping to ensure their stability and minimizing the accumulation of peroxides and degradation products.

Highlights:

- **Accurate**—precise 10% detergent solution in ultrapure water
- **Easy to use**—solution is simple to dispense and dilute
- **Exceptionally pure**—less than 1 µeq/mL peroxides and carbonyls (Tables 2 and 3)
- **Stable**—packaged under inert nitrogen gas in glass ampules or HDPE bottles

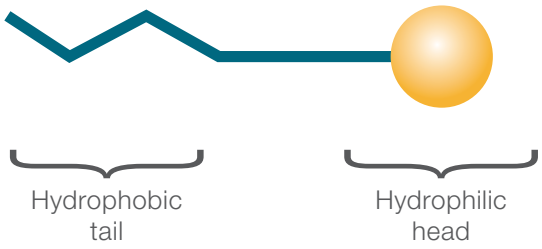


Figure 3. General structure of a detergent molecule.

Table 2. Purity comparison of Tween 20 detergents.

Manufacturer/brand	Peroxide concentration (µeq/mL)	Carbonyl concentration (µeq/mL)
Thermo Scientific	≤0.01	≤0.32
Amresco	0.598	0.399
Anatrace	≤0.01	≤0.32
G-Biosciences	0.718	≤0.32
Millipore	0.037	≤0.32
Roche	0.279	0.445

Peroxide levels were measured using Thermo Scientific™ Pierce™ Quantitative Peroxide Assay Kit, and carbonyl levels were measured using the Brady test for carbonyls.

Table 3. Purity comparison of NP-40 detergents.

Manufacturer/brand	Peroxide concentration (µeq/mL)	Carbonyl concentration (µeq/mL)
Thermo Scientific	≤0.035	≤0.01
Amresco	0.083	0.374
Anatrace	0.053	4.246
G-Biosciences	≤0.035	≤0.01
Millipore	≤0.035	0.042
Roche	0.056	0.021

Peroxide levels were measured using Thermo Scientific™ Pierce™ Quantitative Peroxide Assay Kit, and carbonyl levels were measured using the Brady test for carbonyls.

For more information, or to view additional products, go to **thermofisher.com/detergents**

Protease and phosphatase inhibitors

Broad-spectrum liquid cocktails and tablets for complete protein protection

Protease and phosphatase inhibitor cocktails and tablets are ideal for the protection of proteins during extraction or lysate preparation from primary cells, cultured mammalian cells, animal tissues, plant tissues, yeast cells, or bacterial cells. Formulations are packaged in multiple sizes, and EDTA-free versions are available for assays that are sensitive to divalent cations.

Highlights:

- **Convenient**—ready-to-use, fully disclosed, broad-spectrum formulations available as either liquid cocktails or tablets in multiple pack sizes and with a minimum one year shelf life
- **Complete protection**—combined cocktail available with all-in-one formulations containing both protease and phosphatase inhibitors (Table 4)
- **Compatible**—use directly with Thermo Scientific™ Pierce™ cell lysis buffers or other commercial or homemade detergent-based lysis reagents

Table 4. Components present in Thermo Scientific™ Halt™ Inhibitor Cocktails and Thermo Scientific™ Pierce™ Protease and Phosphatase Inhibitor Tablets.

Inhibitor component	Target (mechanism)	Protease liquid cocktails and tablets	Phosphatase liquid cocktails and tablets	Combined protease and phosphatase liquid cocktails and tablets
AEBSF•HCl	Serine proteases (irreversible)	•		
Aprotinin	Serine protease (reversible)	•		•
Bestatin	Aminopeptidase (reversible)	•		•
E-64	Cysteine (irreversible)	•		•
Leupeptin	Serine and cysteine proteases (reversible)	•		•
Pepstatin	Aspartic acid proteases (reversible)	•		
EDTA*	Metalloproteases (reversible)	•		•
Sodium fluoride	Serine/threonine and acidic phosphatases		•	•
Sodium orthovanadate	Tyrosine and alkaline phosphatases		•	•
β-Glycerophosphate	Serine/threonine phosphatase		•	•
Sodium pyrophosphate	Serine/threonine phosphatase		•	•

* EDTA is not in EDTA-free formulations.

For more information, or to view additional products, go to **thermofisher.com/inhibitorcocktails**

Slide-A-Lyzer dialysis products

Easy-to-handle devices, cassettes, and flasks for secure sample processing

Thermo Scientific™ dialysis units facilitate rapid and trouble-free dialysis of sample volumes from 10 µL to 250 mL. Unlike standard flat tubing, these innovative devices do not require knots or clips that can lead to leaking and sample loss. Thermo Scientific™ Pierce™ 96-Well Microdialysis Plates and Slide-A-Lyzer™ MINI Dialysis Devices are ideal for small volumes, Slide-A-Lyzer™ G3 Dialysis Cassettes are recommended for small to medium volumes, and Slide-A-Lyzer™ Dialysis Flasks are recommended for larger volumes (Table 5).

Highlights:

- **Excellent sample recovery**—low-binding plastics and membranes help minimize sample loss compared to filtration and resin systems, while maximizing dialysis speed (Figures 4 and 5)
- **Convenient**—easy-to-grip design helps simplify sample addition and removal with syringe or pipette
- **Secure**—sealed membranes help prevent leakage that can occur with dialysis tubing and homemade devices
- **Validated**—each device is leak-tested during production

Table 5. High-performance dialysis product selection guide.

Membrane MWCO	10–1,000 µL Pierce Microdialysis Plate	10–2,000 µL Slide-A-Lyzer MINI Dialysis Device	1–125 mL Slide-A-Lyzer G3 Dialysis Cassette	150–250 mL Slide-A-Lyzer Dialysis Flask	15–100 mL Thermo Scientific™ SnakeSkin™ Dialysis Tubing
					
2K	•	•	•	•	NA
3.5K	•	•	•	•	•
7K	NA	•	NA	NA	•
10K	•	•	•	•	•
20K	•	•	•	•	NA

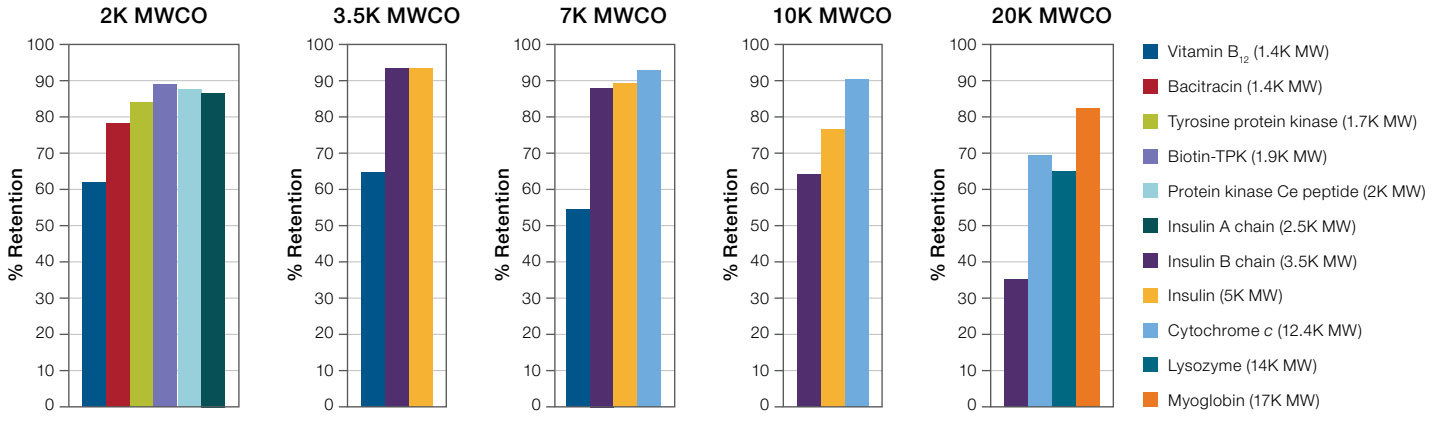


Figure 4. Sample retention by the Thermo Scientific™ Slide-A-Lyzer™ cassette membranes with 2K, 3.5K, 7K, 10K, or 20K molecular weight cutoff (MWCO). Individual proteins or vitamin B_{12} (1 mg/mL) in either saline or 0.2 M carbonate-bicarbonate buffer, pH 9.4, were dialyzed overnight (17 hr) at 4°C. The amount of retentate was estimated using either the Pierce BCA Protein Assay Kit or absorption at 360 nm (for vitamin B_{12}).

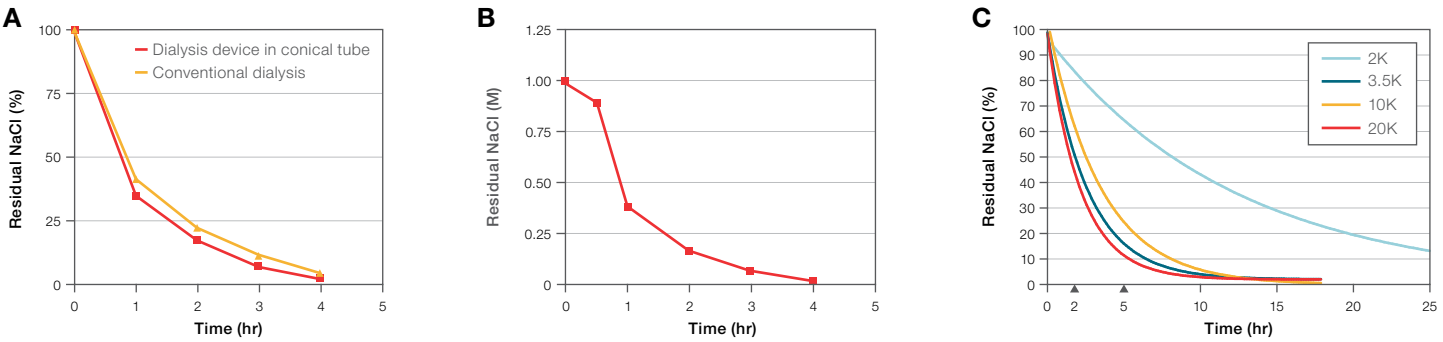
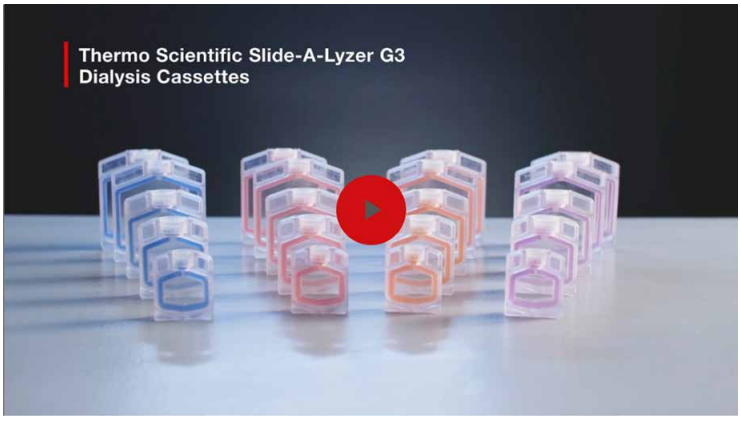


Figure 5. The rate of removal of NaCl using various dialysis products. NaCl removal from samples was determined by measuring the conductivity of the retentate at the indicated times. **(A)** Slide-A-Lyzer MINI Dialysis Device (10K MWCO, 2 mL) vs. conventional dialysis. Bovine serum albumin (BSA) samples (2 mL, 0.25 mg/mL in 1 M NaCl) were dialyzed against 45 mL of water in 50 mL disposable conical tubes on an orbital shaker (300 rpm) at room temperature. The water was changed once after 2 hr. Results are the average of two samples. For conventional dialysis, the samples were dialyzed against 2 L of water in a beaker with stirring. More than 95% of NaCl was removed within 4 hr. **(B)** Samples of 0.1 mL of 0.4 mg/mL cytochrome *c* in 1 M NaCl were dialyzed in the Pierce 96-Well Microdialysis Plate against 1.8 mL of water at room temperature with gentle shaking. The buffer was changed hourly over a 4 hr period. Removal of NaCl was >83% after 2 hr and >99% after 4 hr. **(C)** Proteins in 200 mL samples containing 1 M NaCl were dialyzed at room temperature using Slide-A-Lyzer Dialysis Flasks with 2K, 3.5K, 10K, or 20K MWCOs. The dialysis buffer (4 L) was changed after 2 and 5 hr (triangles) and at 41 hr for the flask with 2K MWCO. More than 95% of NaCl was removed within 8 to 18 hr (41 hr for the flask with 2K MWCO).

How-to video

Watch the video to see how to use Slide-A-Lyzer G3 dialysis cassettes in a typical dialysis protocol.



For more information, or to view additional products, go to **thermofisher.com/dialysis**

Zeba desalting products

Convenient spin formats help ensure rapid desalting with high protein recovery

Thermo Scientific™ Zeba™ desalting products contain proprietary high-performance resins with exceptional desalting and protein recovery characteristics. They can help process even very dilute protein samples with high levels of protein recovery and greater than 95% retention (removal) of salts and other small molecules. The resin is provided in convenient spin columns, plates, and cartridges, for processing sample volumes between 2 µL and 4 mL (Tables 6–8).

Highlights:

- **High performance**—proprietary resin enables excellent protein recovery and efficient contaminant removal (Figure 6)
- **Flexible**—available in spin columns, filter spin plates, and cartridges for a range of needs
- **Fast**—no fraction screening or waiting for protein to emerge by gravity flow
- **Economical**—cost-effective products that offer great performance

Table 6. Zeba desalting products selection guide by format and recommended sample volume.

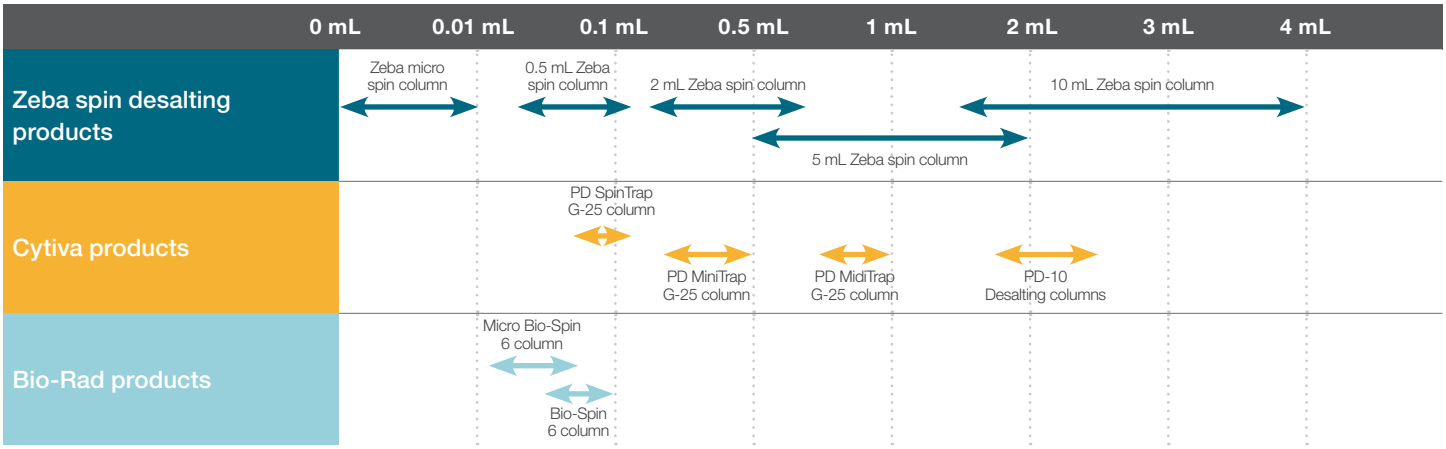
Type	Spin columns					Spin plate	Chromatography columns	
Format	Micro	0.5 mL	2 mL	5 mL	10 mL	96-well	1 mL	5 mL
								
Resin bed	75 µL	0.5 mL	2 mL	5 mL	10 mL	550 µL	1 mL	5 mL
Sample volume (7K MWCO)	2–12 µL	30–130 µL	200–700 µL	500–2,000 µL	700–4,000 µL	20–100 µL	50–250 µL	100–1,500 µL
Sample volume (40K MWCO)	5–14 µL	70–200 µL	200–900 µL	300–2,000 µL	1,000–4,000 µL	20–100 µL	NA	NA

Table 7. Thermo Scientific™ Zeba™ resin selection guide by protein recovery and small molecule removal.

	7K MWCO		40K MWCO	
Size	Recovery	Removal	Recovery	Removal
Peptide/protein <7 kDa	NR*		NR	
Protein 7–13 kDa	++		++	
Protein 14–20 kDa	+++		+++	
Protein 20–150 kDa	+++		+++	
Molecule <500 Da		+++		+++
Molecule 600–1,200 Da		++		+++
Molecule 1,200–1,500 Da		+		++
Molecule >1,500–2,000 Da		NR		+

* NR: No recovery or removal.

Table 8. Comparison of recommended sample volume capacities of common spin desalting products.



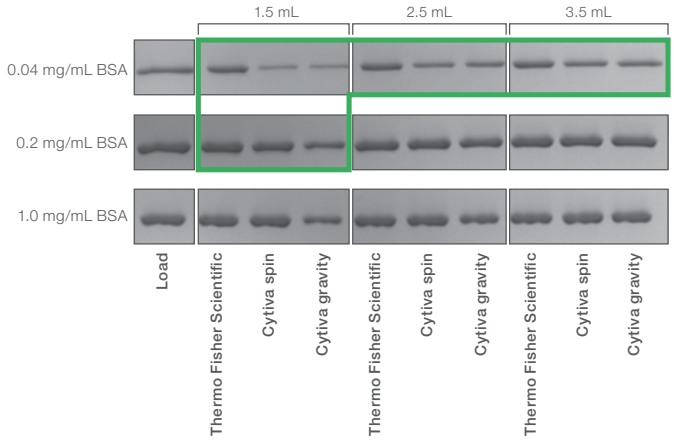


Figure 6. Zeba Spin Desalting Columns result in high protein recovery while providing minimal sample dilution over a wider range of sample concentrations and volumes compared to alternative products. Zeba Spin Desalting Columns, 10 mL (7K MWCO), and Cytiva PD-10 Columns were used to desalt 1.5, 2.5, and 3.5 mL BSA samples at concentrations of 0.04, 0.2, and 1 mg/mL. Desalting was performed according to the manufacturers' recommended protocols; both the spin and gravity protocols were used for the Cytiva PD-10 Columns. Protein recovery was analyzed by SDS-PAGE. For each electrophoresis gel, an aliquot of starting sample equal to 1 µg of BSA was loaded in lane 1 as the loading control; all other desalted samples were loaded in the gel at the same volume as the loading control. Differences in intensity between lanes are a combination of protein recovery and sample dilution caused by desalting. The largest differences in recovery and concentration were noticed in the outlined area.

For more information, or to view additional products, go to **thermofisher.com/desalting**

Protein concentrators

Easy-to-use devices for rapid and efficient concentration

Thermo Scientific™ Pierce™ Protein Concentrators are easy-to-use centrifugal devices that provide fast processing and excellent recovery of protein samples. These disposable ultrafiltration devices are available in four volume capacities (0.5, 6, 20, and 100 mL) and contain a polyethersulfone (PES) membrane in five distinct molecular weight cutoffs (MWCOs): 3K, 5K, 10K, 30K, 50K, and 100K, for the concentration, desalting, and buffer exchange of biological samples, such as tissue culture media and chromatography fractions, or to remove unincorporated label following protein bioconjugation reactions.

Highlights:

- **Rapid processing**—unique design minimizes membrane fouling
- **High recovery**—retain >90% of protein samples while removing contaminants or exchanging buffers (Figure 7)
- **Convenient**—clear markings, wide sample chamber, and removable filtrate chamber make handling simple and easy

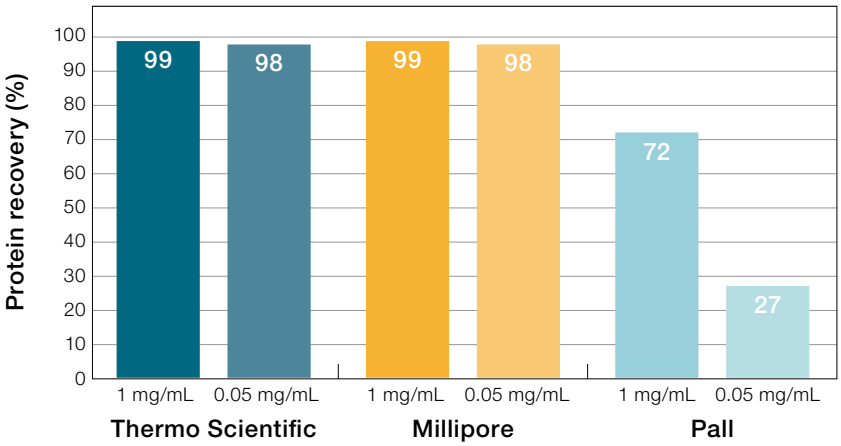


Figure 7. Pierce Protein Concentrators provide exceptional recovery of low-concentration samples. Samples of aprotinin (~6.5 kDa) were centrifuged in a Pierce Protein Concentrator, PES (3K MWCO, 0.5 mL), and other suppliers' concentrators at 15,000 x *g* until the sample volume was 1/15 of the original volume. Protein recovery was determined using the Pierce BCA Protein Assay Kit.

For more information, or to view additional products, go to **thermofisher.com/concentrators**

Protein quantitation products

Protein assays

Accurately quantifying total protein concentration is a key step in most experiments and workflows involving isolation, separation, and analysis of proteins by biochemical methods. Depending on the accuracy required and the amount and purity of the protein available, different methods are appropriate for determining protein concentration. Colorimetric and fluorescent reagent-based protein assay techniques are available. Protein is added to the reagent, producing a color change or fluorescent readout in proportion to the amount added. A standard curve consisting of known concentrations of a purified protein is used to determine protein concentration. Because of the diversity of protein samples, careful consideration should be given to the choice of assay.

Important criteria for choosing an assay include:

- Compatibility with the sample type and buffer components
- Assay working range and required sample volume
- Protein-to-protein uniformity
- Speed and convenience for the number of samples to be tested
- Availability of necessary equipment to measure the assay output

Multiple formulations for flexible and accurate quantitation

We offer numerous colorimetric and fluorescent assays for detection and quantitation of total protein to meet the sensitivity and compatibility requirements of your samples (Table 9). In addition, we offer easy-to-use colorimetric or fluorescent peptide assays that are designed specifically to improve the sensitivity and reproducibility of peptide mixture quantitation.

Table 9. Protein assay guide.

	Thermo Scientific™ Pierce™ Rapid Gold BCA Protein Assay Kit	Thermo Scientific™ Pierce™ BCA Protein Assay Kit	Thermo Scientific™ Pierce™ Coomassie Plus (Bradford) Assay Kit	Invitrogen™ Qubit™ Protein BR Assay Kit	Invitrogen™ NanoOrange™ Protein Quantitation Assay
Assay highlights	5 min room temperature BCA assay	Detergent-compatible assay with linear curve and high accuracy	Reducing-agent compatible, rapid assay	Rapid quantitation for broad range of protein concentrations	Best for dilute samples or samples with limited volume
Minimum sample volume	10 µL	25 µL	10 µL	10 µL	1 µL
Working range	125–2,000 µg/mL	20–2,000 µg/mL	100–1,500 µg/mL	100–20,000 µg/mL	0.01–10 µg/mL
Compatibility	Detergents	Detergents	Reducing agents	Detergents and reducing agents	Reducing agents
Incubation temperature	RT	37°C	RT	RT	90–95°C
Assay incubation time	5 min	30 min	5 min	10 min	10 min
Assay wavelength	480 nm	562 nm	595 nm	470/570 nm	470/570 nm
Required equipment	Spectrophotometer or absorbance microplate reader	Spectrophotometer or absorbance microplate reader	Spectrophotometer or absorbance microplate reader	Qubit 4 Fluorometer	Fluorometer or fluorescence microplate reader

For a full list of available protein concentration assays, visit **thermofisher.com/proteinassays**

Accurate detergent-compatible Pierce BCA assays

BCA protein assays have a unique advantage over Bradford (Coomassie dye–based) assays, as they are compatible with samples that contain up to 5% surfactants (detergents) and are affected much less by protein compositional differences, providing greater protein-to-protein uniformity and accuracy (Figure 8).

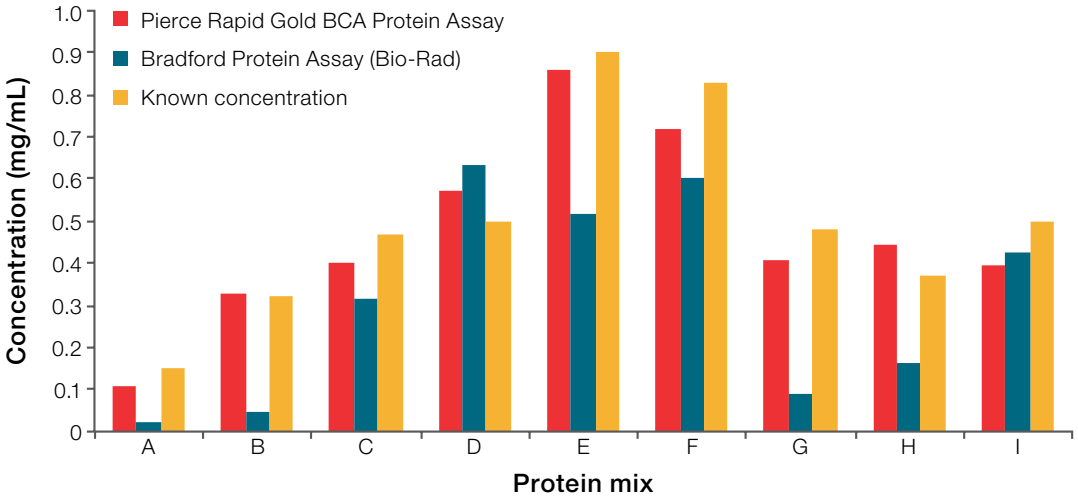


Figure 8. Accuracy of the Pierce Rapid Gold BCA Protein Assay and Bradford Protein Assay with known protein mixes. Both assays were conducted according to the respective manufacturers' protocols, in a microplate format. For the Bradford assay, 10 µL of the BSA sample was added to 200 µL of the Bradford working reagent and incubated at room temperature for 5 minutes. For the Pierce Rapid Gold BCA Protein Assay, 20 µL of sample was added to 200 µL of Rapid Gold BCA working reagent and incubated at room temperature for 5 minutes. Known concentrations were based on manufacturers' indicated concentrations and confirmed by absorbance at 280 nm.

Broad dynamic range of the Qubit Protein BR Assay

The Invitrogen™ Qubit™ Protein BR Assay is a fluorometric assay that combines accuracy, compatibility, and ease of use to detect total protein concentrations over a broad dynamic range. The broad linear response allows accurate determination of unknown protein concentrations and provides a higher dynamic range than other standard protein assays (Figure 9). The Qubit Protein BR Assay can be used to detect protein concentrations from 100 to 20,000 µg/mL and is compatible with many commonly used detergents and reducing agents. This allows most samples to be used neat (undiluted), eliminating the guesswork and dilution steps that accompany many protein quantitation methods.

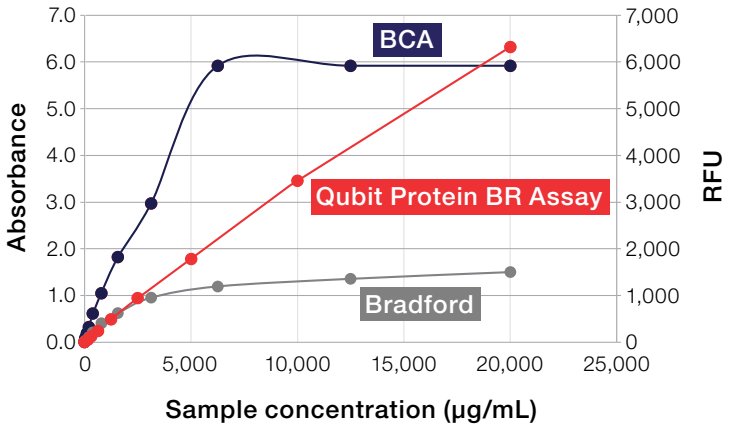


Figure 9. Standard curves for protein quantitation assays. Purified bovine serum albumin (BSA) in 0.9% saline (0–20 mg/mL) was used to generate standard curves for the Qubit Protein BR Assay, Pierce BCA Protein Assay, and the Bradford assay. Assays were conducted following the manufacturers' protocols. The BCA and Bradford assays were performed in microplate format.

Qubit 4 Fluorometer

The Qubit Protein BR Assay is optimized for use with the Invitrogen™ Qubit™ 4 Fluorometer. The Qubit 4 Fluorometer provides the combination of a user-friendly fluorometer and capability to run highly sensitive fluorescence-based quantitation assays. The Qubit 4 Fluorometer is a small, economical instrument designed to work seamlessly with Invitrogen™ Qubit™ assay kits for routine protein, DNA, and RNA quantitation, and RNA integrity and quality assessment. All settings and calculations are performed directly on the instrument and can be transferred by USB drive, USB cable, or by Wi-Fi. The system is simple, fast, and easy to use, yet enables consistently accurate results for subsequent applications. Only small sample volumes of 1–20 µL are required for all assays.

Multiskan SkyHigh Microplate Spectrophotometer

The Thermo Scientific™ Multiskan™ SkyHigh Microplate Spectrophotometer provides ease of use for all photometric protein quantitation measurements. The touchscreen models offer the flexibility to use the system as a stand-alone instrument, or in conjunction with Thermo Scientific™ SkanIt™ PC software.

For direct 280 nm measurements, the system provides ready-made protocols for path length correction, which normalize the absorbance values of microvolumes to values measured in standard cuvettes. The colorimetric dye measurement is simple to perform with either internal user interface pre-built sessions or by downloading the pre-built session with all the data handling from the SkanIt Cloud Library.

For more details, visit **thermofisher.com/platereaders**

Test your protein research knowledge

Question: Which of the following are important considerations when selecting an appropriate protein quantitation assay?

A. Sample type and buffer component compatibility

B. Assay working range and required sample volume

C. Speed and convenience

D. All of the above

Answer: D

Ordering information

Product	Quantity	Cat. No.
Extraction		
Protein extraction reagents and subcellular fractionation kits		
M-PER Mammalian Protein Extraction Reagent	250 mL	78501
T-PER Tissue Protein Extraction Reagent	500 mL	78510
Pierce IP Lysis Buffer	100 mL	87787
RIPA Lysis and Extraction Buffer	250 mL	89901
Pierce IP Lysis Buffer	100 mL	87787
NE-PER Nuclear and Cytoplasmic Extraction Reagents	75 mL	78835
Mem-PER Plus Membrane Protein Extraction Kit	300 mL	89842
Mitochondria Isolation Kit for Cultured Cells	115 mL	89874
Subcellular Protein Fractionation Kit for Cultured Cells	35 mL	78840
Subcellular Protein Fractionation Kit for Tissues	115 mL	87790
Syn-PER Synaptic Protein Extraction Reagent	100 mL	87793
Pierce Cell Surface Protein Isolation Kit	8 reactions	89881
Lysosome Enrichment Kit for Tissues and Cultured Cells	230 mL	89839
Y-PER Yeast Protein Extraction Reagent	500 mL	78990
I-PER Insect Cell Protein Extraction Reagent	250 mL	89802
Pierce Plant Total Protein Extraction Kit	50 preparations	A44056
B-PER Complete Bacterial Protein Extraction Reagent	250 mL	89821
Pierce Universal Nuclease for Cell Lysis	25 kU	88701
	100 kU	88702
Pierce GPCR Extraction and Stabilization Reagent	100 mL	A43436
N-PER Neuronal Protein Extraction Reagent	100 mL	87792

To view additional pack sizes and products, go to **thermofisher.com/proteinextraction**

Detergents		
Tween 20 Surfact-Amps Detergent Solution	6 x 10 mL	28320
	50 mL	85113
Tween 80 Surfact-Amps Detergent Solution	6 x 10 mL	28328
	50 mL	28329
Triton X-100 Surfact-Amps Detergent Solution	6 x 10 mL	28314
	50 mL	85111
Triton X-114 Surfact-Amps Detergent Solution	6 x 10 mL	28332
NP-40 Surfact-Amps Detergent Solution	6 x 10 mL	28324
	50 mL	85124
Brij-35 Surfact-Amps Detergent Solution	6 x 10 mL	28316
	50 mL	85117
Brij-58 Surfact-Amps Detergent Solution	6 x 10 mL	28336

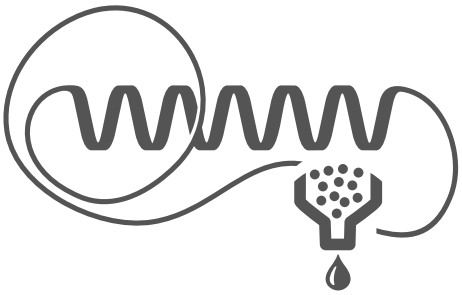
To view additional pack sizes and products, go to **thermofisher.com/detergents**

Product	Quantity	Cat. No.
Inhibitor cocktails and tablets		
Halt Protease Inhibitor Single-Use Cocktail (100X)	24 x 100 µL	78430
Halt Protease Inhibitor Cocktail (100X)	1 mL	87786
	5 mL	78429
Halt Protease Inhibitor Single-Use Cocktail, EDTA-free (100X)	24 x 100 µL	78425
Halt Protease Inhibitor Cocktail, EDTA-free (100X)	1 mL	87785
	5 mL	78437
Pierce Protease Inhibitor Mini Tablets	30 tablets	A32953
Pierce Protease Inhibitor Tablets	20 tablets	A32963
Pierce Protease Inhibitor Mini Tablets, EDTA-free	30 tablets	A32955
Pierce Protease Inhibitor Tablets, EDTA-free	20 tablets	A32965
Halt Phosphatase Inhibitor Single-Use Cocktail (100X)	24 x 100 µL	78428
Halt Phosphatase Inhibitor Cocktail (100X)	1 mL	78420
	5 x 1 mL	78426
Pierce Phosphatase Inhibitor Mini Tablets	20 tablets	A32957
Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X)	24 x 100 µL	78442
Halt Protease and Phosphatase Inhibitor Cocktail (100X)	1 mL	78440
Halt Protease and Phosphatase Inhibitor Single-Use Cocktail, EDTA-free (100X)	24 x 100 µL	78443
Halt Protease and Phosphatase Inhibitor Cocktail, EDTA-free (100X)	1 mL	78441
Pierce Protease and Phosphatase Inhibitor Mini Tablets	20 tablets	A32959
Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-free	20 tablets	A32961
To view additional pack sizes and products, go to **thermofisher.com/inhibitorcocktails**		
Cleanup		
Dialysis devices, cassettes, and flasks		
Slide-A-Lyzer MINI Dialysis Device, 2K MWCO, 0.1 mL	50 devices	69580
Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 0.1 mL	50 devices	69550
Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 0.1 mL	50 devices	69570
Slide-A-Lyzer MINI Dialysis Device, 20K MWCO, 0.1 mL	50 devices	65950
Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 0.5 mL	25 devices	88400
Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 0.5 mL	25 devices	88401
Slide-A-Lyzer MINI Dialysis Device, 3.5K MWCO, 2 mL	25 devices	88403
Slide-A-Lyzer MINI Dialysis Device, 10K MWCO, 2 mL	25 devices	88404
Slide-A-Lyzer Dialysis Flask, 3.5K MWCO, 230 mL	4 flasks	87761
Slide-A-Lyzer Dialysis Flask, 10K MWCO, 250 mL	4 flasks	87762
Pierce 96-Well Microdialysis Plate, 0.1 mL, 10K MWCO	1 plate	88260
Pierce 96-Well Microdialysis Plate, 0.3 mL, 10K MWCO	1 plate	A50469
Pierce 48-Well Microdialysis Plate, 1 mL, 10K MWCO	1 plate	A50470
Slide-A-Lyzer G3 Dialysis Cassettes, 2K MWCO, 3 mL	10 cassettes	A52961
Slide-A-Lyzer G3 Dialysis Cassettes, 3.5K MWCO, 3 mL	10 cassettes	A52966
Slide-A-Lyzer G3 Dialysis Cassettes, 10K MWCO, 3 mL	10 cassettes	A52971
Slide-A-Lyzer G3 Dialysis Cassettes, 20K MWCO, 3 mL	10 cassettes	A52976
Slide-A-Lyzer G3 Dialysis Cassettes, 3.5K MWCO, 15 mL	8 cassettes	A52967
Slide-A-Lyzer G3 Dialysis Cassettes, 10K MWCO, 15 mL	8 cassettes	A52972
Slide-A-Lyzer G3 Dialysis Cassettes, 3.5K MWCO, 30 mL	6 cassettes	A52968
Slide-A-Lyzer G3 Dialysis Cassettes, 10K MWCO, 30 mL	6 cassettes	A52973
Slide-A-Lyzer G3 Dialysis Cassettes, 3.5K MWCO, 70 mL	6 cassettes	A52969
Slide-A-Lyzer G3 Dialysis Cassettes, 10K MWCO, 70 mL	6 cassettes	A52974
Slide-A-Lyzer G3 Dialysis Cassettes, 3.5K MWCO, 125 mL	6 cassettes	A52970
Slide-A-Lyzer G3 Dialysis Cassettes, 10K MWCO, 125 mL	6 cassettes	A52975
To view additional pack sizes and MWCOs, go to **thermofisher.com/dialysis**		

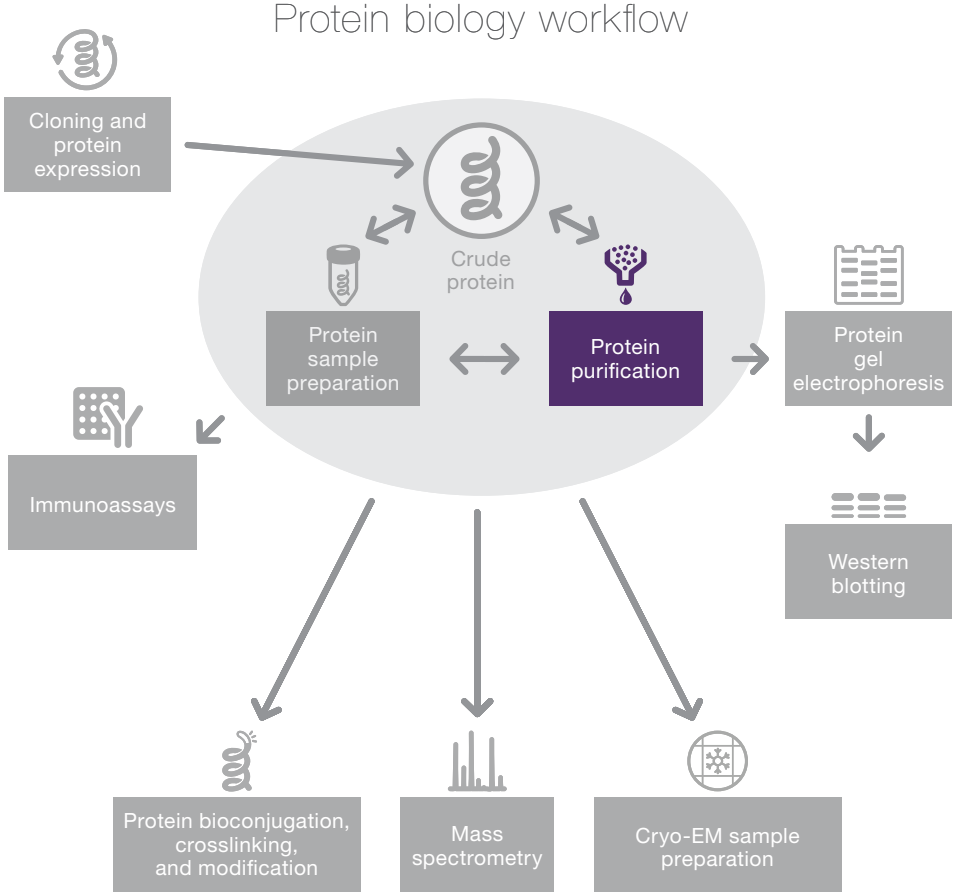
Product	Quantity	Cat. No.
Desalting products		
Zeba Micro Spin Desalting Columns, 7K MWCO, 75 µL	25 columns	89877
Zeba Spin Desalting Columns, 7K MWCO, 0.5 mL	25 columns	89882
Zeba Spin Desalting Columns, 40K MWCO, 0.5 mL	25 columns	87766
Zeba Spin Desalting Columns, 7K MWCO, 2 mL	25 columns	89890
Zeba Spin Desalting Columns, 40K MWCO, 2 mL	25 columns	87769
Zeba Spin Desalting Columns, 7K MWCO, 5 mL	25 columns	89892
Zeba Spin Desalting Columns, 7K MWCO, 10 mL	25 columns	89894
Zeba 96-Well Spin Desalting Plates, 7K MWCO	2 plates	89807
Zeba Desalting Chromatography Cartridges, 7K MWCO, 1 mL	5 cartridges	89934
Zeba Desalting Chromatography Cartridges, 7K MWCO, 5 mL	5 cartridges	89935
Zeba Micro Spin Desalting Columns, 40K MWCO, 75 µL	25 columns	87764
To view additional pack sizes and MWCOs, go to **thermofisher.com/desalting**		
Protein concentrators		
Pierce Protein Concentrators PES, 3K MWCO, 0.5 mL	25 pack	88512
Pierce Protein Concentrators PES, 10K MWCO, 0.5 mL	25 pack	88513
Pierce Protein Concentrators PES, 30K MWCO, 0.5 mL	25 pack	88502
Pierce Protein Concentrators PES, 100K MWCO, 0.5 mL	25 pack	88503
Pierce Protein Concentrators PES, 3K MWCO, 2–6 mL	24 pack	88515
Pierce Protein Concentrators PES, 10K MWCO, 2–6 mL	24 pack	88517
Pierce Protein Concentrators PES, 30K MWCO, 2–6 mL	24 pack	88522
Pierce Protein Concentrators PES, 100K MWCO, 2–6 mL	24 pack	88524
Pierce Protein Concentrators PES, 10K MWCO, 5–20 mL	24 pack	88528
Pierce Protein Concentrators PES, 10K MWCO, 20–100 mL	24 pack	88535
Pierce Protein Concentrators PES, 50K MWCO, 5–20 mL	24 pack	88541
Pierce Protein Concentrators PES, 50K MWCO, 20–100 mL	4 pack	88542
To view additional pack sizes and MWCOs, go to **thermofisher.com/concentrators**		
Protein quantitation		
Protein assays		
Pierce Rapid Gold BCA Protein Assay Kit	250 mL	A53226
	500 mL	A53225
Pierce BCA Protein Assay Kit	500 mL	23227
	1 L	23225
Pierce Coomassie Plus (Bradford) Assay Kit	950 mL	23236
Qubit Protein BR Assay Kit	100 assays	A50668
	500 assays	A50669
Qubit Protein BR Starter Kit	1 instrument and kit	A51292
NanoOrange Protein Quantitation Kit	1 kit	N6666
To view additional pack sizes and products, go to **thermofisher.com/proteinassays**		

Protein purification

Protein purification is essential to the study of protein function, structure, and interactions. A variety of methods can be used to enrich or purify a protein of interest from other proteins and components in a crude cell lysate or other sample. Common techniques include ion-exchange and affinity chromatography and immunoprecipitation.



Protein biology workflow



Protein purification and immunoprecipitation

Ion exchange (IEX) and affinity chromatography are two strategies commonly used for partial or one-step protein purification. IEX chromatography enables the separation of proteins based on the protein charge at a particular pH. Since multiple proteins may have similar charges, IEX chromatography generally enables only partial purification of a protein of interest. Affinity chromatography is enabled by specific binding of a protein to an immobilized ligand. Because the protein of interest is tightly bound, contaminants can be removed through wash steps, and the bound protein can be stripped (eluted) from the support in a highly purified form. Affinity purification produces higher protein yields and requires fewer steps than other purification methods.

Immunoprecipitation (IP) is small-scale affinity purification of antigens using a specific antibody that is immobilized to a solid support, and is one of the most widely used methods for isolation of proteins and other biomolecules from cell or tissue lysates for the purpose of subsequent detection by western blotting or other techniques. Other similar techniques include co-immunoprecipitation (co-IP) and pull-down, which are similar to IP except that the target antigen is used to co-precipitate its binding partner(s) or associated protein complex from the lysate.

Protein purification products

High-performance resins and magnetic beads for maximum protein yield

Our protein purification portfolio offers a broad range of products for ion exchange and affinity-based isolation of proteins and antibodies in microgram to kilogram quantities. Strong anion or cation exchange resins provide an intermediate level of purification during multi-step isolation or act as a polishing step during the final stages of purification. Biotinylated or recombinant proteins can be conveniently captured using avidin-based or affinity tag–based binding supports. Customized protein purification can be achieved by immobilizing ligands to the appropriate support. Accessory products are available for increased convenience, including disposable columns and binding and elution buffers. Rapid protein screening or IP, co-IP, and pull-down applications can be completed utilizing magnetic bead–based resins and kits, as described on pages 35–37.

Highlights:

- **Broad product selection**—strong ion exchange and affinity supports for purification and enrichment of proteins and antibodies; affinity ligands enable one-step purification of recombinant and biotinylated proteins, while activated supports provide a platform for custom protein immobilization (Table 1)
- **High performance**—resins are designed to maximize protein yield and reduce background
- **More formats**—magnetic beads, loose resins, FPLC cartridges, and 96-well filter plates enable protein purification from screening and small-scale phases to process-scale purification (Table 2)
- **Economical**—pricing that is similar to or better than products from other leading suppliers

Table 1. Overview of ion exchange, affinity, and activated supports.

Application	Purity level	Ligand and/or chemistry	Base bead type	Packaging options
Ion exchange purification	Medium to high (application-specific)	Strong anion exchange	POROS	Loose resin
		Strong cation exchange		
Antibody purification	High	Protein A, protein G, protein A/G	Agarose, magnetic beads, magnetic agarose, POROS	Loose resins or beads, spin columns and kits, chromatography cartridges, 96-well spin plates
		Protein L	Agarose, magnetic beads	
		Melon Gel	Agarose	
Fusion protein purification	High	Ni-NTA, Ni-IDA, cobalt, glutathione	Agarose, Superflow, magnetic beads, magnetic agarose	Loose resins or beads, spin columns and kits, chromatography cartridges, 96-well spin plates
		Anti–c-Myc, anti-HA, anti-FLAG	Agarose, UltraLink magnetic beads	Loose resins or beads, kits
Biotin affinity purification	High	Avidin, streptavidin, NeutrAvidin, monomeric avidin	Agarose, magnetic beads	Loose resins, spin columns and kits, chromatography cartridges, 96-well spin plates
Protein immobilization	High	Amine-reactive, sulfhydryl-reactive, carbonyl-reactive, carboxyl-reactive	Agarose	Loose resins or dry powder
		Epoxy, tosyl-activated, carboxylic acid, amine	Magnetic beads	Loose beads

Table 2. Select your resin based on purification scale and application.

Scale	High-throughput screening	High-throughput batch	Batch	Pilot	Process
Description	Small scale, automation compatible	Lab or bench scale	Lab or bench scale	Scale-up desired	Production scale
Yield	Microgram	Milligram	Milligram	Gram	Kilogram
Format	Magnetic particle processor	Magnetic particle processor, 96-well spin plate (agarose)	Gravity flow, spin column (agarose), fast protein liquid chromatography (FPLC) at low flow rates	FPLC at medium flow rates	FPLC at high flow rates
Application	High-throughput screening, interaction studies (IP, co-IP, pull-down), mutational analysis	High-throughput screening, interaction studies (IP, co-IP, pull-down), mutational analysis requiring mg scale	Functional assays, structural analysis	Structural analysis, intermediate-scale production	Bulk production
Recommended resin type	 Magnetic bead (1–2.8 µm)				
		 Magnetic agarose (10–40 µm)			
		 Agarose (45–165 µm)			
			 Superflow (45–165 µm)		
			 UltraLink resin (50–80 µm)		
			 POROS resin (50 µm)		

Ion exchange chromatography resins and membranes

We offer strong cation exchange (SCX) and strong anion exchange (SAX) resins composed of rigid polymeric beads with covalent surface chemistries. The robust manufacturing process yields outstanding physical and chemical stability, allowing easier handling and packing. These high-capacity chromatography media are designed to deliver excellent separation and scale-up capabilities. See Table 3 for recommended products based on application.

Thermo Scientific™ Pierce™ Strong Cation or Anion Exchange Spin Columns are membrane-based centrifugal devices that eliminate the need for column packing, allow multiple samples to be processed simultaneously, and are ideal for working with low-volume buffer solutions.

Table 3. Strong ion exchange purification selection guide.

Chemistry	Salt tolerance	Recommended product	High-throughput screening	High-throughput batch	Batch	Pilot	Process
Strong anion exchange	≤25 mM	Pierce Strong Anion Exchange Spin Columns	•	•			
	≤150 mM	POROS HQ resin			•	•	•
	≤50 mM	POROS XQ resin			•	•	•
Strong cation exchange	≤25 mM	Pierce Strong Cation Exchange Spin Columns	•	•			
	≤150 mM	POROS XS resin			•	•	•

Affinity chromatography resins

Our broad menu of resins and formats offers many options for single-step purification of biotinylated or recombinant proteins and antibodies. In addition, customized purification solutions can be designed by the covalent attachment of a ligand to one of our activated supports. Accessory products for all aspects of purification, including disposable columns and binding and elution buffers, are also available.

Antibody purification

Proteins A, G, A/G, and L have unique properties, which make each one suitable for different types of antibody targets (e.g., antibody subclass or animal species). These and other ligands enable purification of general immunoglobulins from a crude sample (Table 4). Depending on the sample source, an antigen-specific antibody may account for only a small portion of the total immunoglobulin in the sample. For example, generally only 2–5% of total IgG in mouse serum is specific for the antigen used to immunize the animal.

Thermo Scientific™ POROS™ MabCapture™ Select resins demonstrate outstanding binding capacity at a range of flow rates, making them suitable for large-scale bioprocessing needs (Figure 1).

Table 4. Antibody purification selection guide for Invitrogen™ and Thermo Scientific™ products.

Mode	Description	Recommended product	High-throughput screening	High-throughput batch	Batch	Pilot	Process
Negative selection	Removal of all non-immunoglobulin proteins	Melon Gel			•		
IgG enrichment	Immobilized immunoglobulin-binding proteins, to selectively remove IgG from a serum sample	Dynabeads Protein A magnetic beads	•				
		Pierce High Capacity Protein A MagBeads, alkali stable		•			
		MabCaptureC High Capacity Protein A Resin, alkali stable		•			
		Pierce High Capacity Ni-IMAC MagBeads and Resin, EDTA compatible		•			
		Pierce Protein A Plus Agarose			•		
		POROS MabCapture A Select Resin			•	•	•
		Dynabeads Protein G magnetic beads	•				
		Pierce Protein G Plus Agarose			•		
		POROS MabCapture G Select Resin			•	•	•
		Pierce Protein A/G Magnetic Beads	•				
		Pierce Protein A/G Magnetic Agarose		•			
		Pierce Protein A/G Plus Agarose			•		
		POROS MabCapture A/G Select Resin			•	•	•
		Pierce Protein L Magnetic Beads	•				
		Pierce Protein L Agarose			•		
IgG enrichment	Thiophilic adsorption	Pierce Thiophilic Adsorbent			•		
IgM enrichment	Immobilized mannan-binding protein (MBP)	Pierce Mannan Binding Protein Agarose			•		
IgA enrichment	Immobilized jacalin, a D-galactose–binding lectin	Pierce Jacalin Agarose			•		

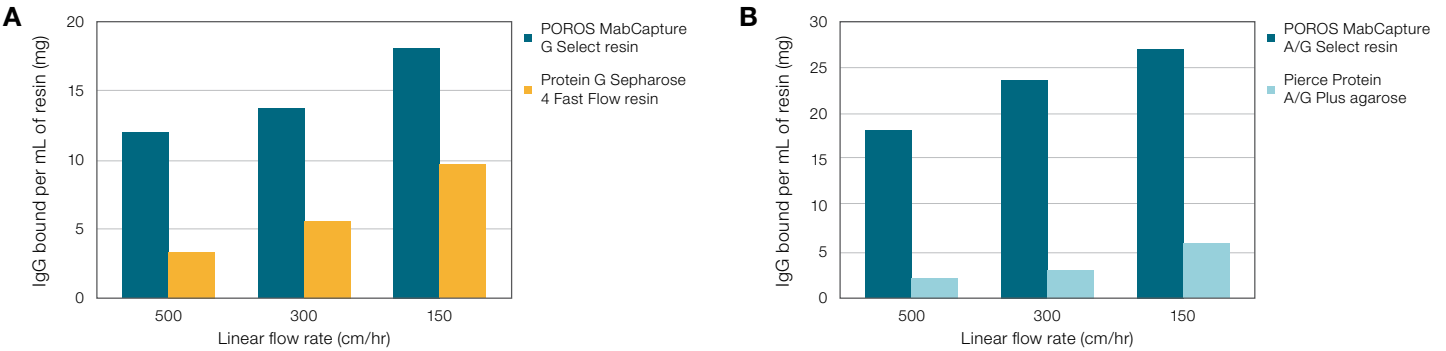


Figure 1. Comparison of dynamic binding capacity vs. flow rate. Each column (0.5 cm ID x 5 cm) was packed with 1 mL of resin and was challenged with human IgG (1 mg/mL) at flow rates of 500, 300, or 150 cm/hr (corresponding to residence times of 0.3, 1, and 2 min, respectively). The dynamic binding capacity (total protein loaded) was determined at 10% breakthrough. **(A)** Comparison between Thermo Scientific™ POROS™ MabCapture™ G Select and Cytiva™ Protein G Sepharose™ 4 Fast Flow resins. **(B)** Comparison between Thermo Scientific™ POROS™ MabCapture™ A/G Select and Thermo Scientific™ Pierce™ Protein A/G Plus resins.

Recombinant protein purification

We offer a variety of resins for the purification of recombinant proteins. These resins are available in multiple formats to accommodate a variety of needs, from high-throughput screening to batch and pilot-scale purification. Superflow resins have undergone extensive chemical characterization. We have ligands targeting a variety of fusion tags, including 6xHis, GST, FLAG™ epitope, c-Myc, and HA (Table 5).

Table 5. Thermo Scientific™ recombinant protein purification selection guide.

Tag	Ligand	Features	Recommended product	High-throughput screening	High-throughput batch	Batch	Pilot
DYKDDDDK (FLAG)	Anti-FLAG	Immobilized antibody	Pierce Anti-DYKDDDDK Magnetic Agarose		•		
			Pierce Anti-DYKDDDDK Affinity Resin (UltraLink resin)			•	•
c-Myc	Anti–c-Myc	Immobilized antibody	Pierce Anti–c-Myc Magnetic Beads	•			
			Pierce Anti–c-Myc Agarose (Superflow)			•	•
HA	Anti-HA	Immobilized antibody	Pierce Anti-HA Magnetic Beads	•			
			Pierce Anti-HA Agarose			•	
6xHis	Ni-NTA or Ni-IDA	Higher protein yield	Pierce Ni-NTA Magnetic Agarose Beads		•		
			ProBond Nickel Chelating Resin			•	
			HisPur Ni-NTA Agarose Resin			•	
			HisPur Ni-NTA Superflow Resin				•
	Cobalt	Higher protein purity	Dynabeads His-Tag Isolation Magnetic Beads	•			
			HisPur Cobalt Agarose Resin			•	
			HisPur Cobalt Superflow Resin				•
GST	Glutathione	Solubility and purification tag	Pierce Glutathione Magnetic Agarose Beads		•		
			Pierce Glutathione Agarose			•	
			Pierce Glutathione Superflow Agarose				•

For more information, or to view additional products, go to **thermofisher.com/proteinpurification**

Immunoprecipitation (IP), co-IP, and pull-down using magnetic beads

Fast, reproducible sample processing with high yield and low nonspecific binding

Magnetic beads are the most rapidly growing method for IP and pull-down assays because they are faster, easier, and more efficient for pulling down proteins than nonmagnetic methods. We offer a wide variety of conjugated magnetic beads, including the highly referenced Invitrogen™ Dynabeads™ magnetic beads and the economical Thermo Scientific™ Pierce™ magnetic beads or magnetic agarose, to meet most application and budget needs.

Highlights:

- **Low background**—little to no nonspecific binding, and no preclearing
- **Highly sensitive**—magnetic beads are the ideal choice for sensitive applications, such as IP of low-abundance proteins
- **Antibody savings**—all binding occurs on the smooth outer surface of the beads, conserving precious antibodies and providing a solution that is cost-efficient per sample
- **Fast and easy**—magnetic beads offer a rapid IP protocol, with no centrifugation or preclearing steps (Figure 2)
- **Flexible**—products for IP, co-IP, and pull-down assays; ideal for both manual and automated protocols
- **Compatible**—magnetic beads can be used in multiple workflows, including western blotting, mass spectrometry, and qPCR (for ChIP analysis)
- **Automation ready**—Dynabeads products for IP can be optimized with a Thermo Scientific™ KingFisher™ purification system to decrease hands-on preparation time and increase sample output (Figures 3 and 4)

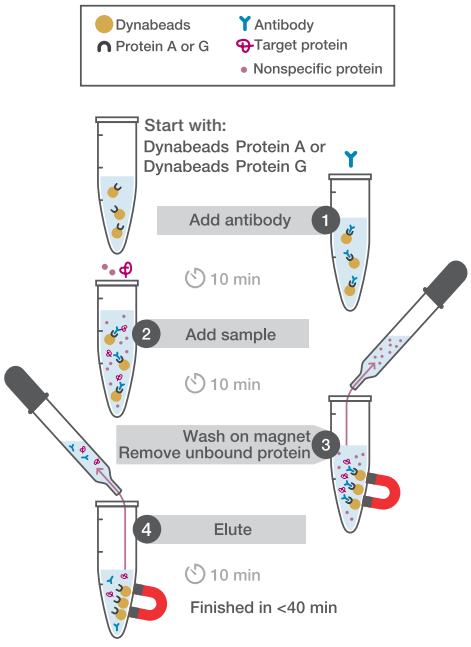


Figure 2. Immunoprecipitation in <40 minutes. Dynabeads magnetic beads precoupled with protein A or protein G act as a suspendable solid support that can be fixed by the use of a magnet. This allows for simple and efficient antibody capture, followed by immunoprecipitation of pure target peptides, proteins, protein complexes, or other antigens.

Figure 3. Thermo Scientific™ KingFisher™ Apex instrument.

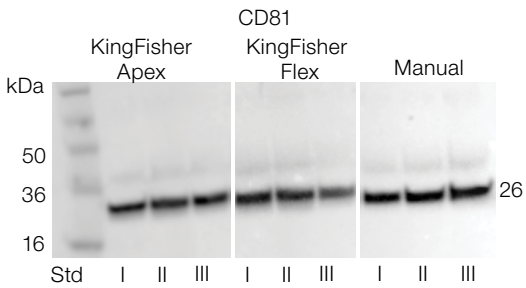


Figure 4. IP of CD81 using manual methods vs. Thermo Scientific™ KingFisher™ Flex and Apex instruments. Jurkat cells expressing CD81 were lysed and incubated with Dynabeads Protein G coated with anti-CD81 antibody using manual and automated methods in triplicate. The samples were prepared for electrophoresis and western blotting, and labeling was performed using the anti-CD81 antibody.

For more information about the KingFisher automation system, or to schedule a demo, go to **thermofisher.com/kingfisherapex**

The optimal product for IP, co-IP, and pull-down assays depends on the antibody or recombinant protein in the application (Table 6).

Table 6. Choose your isolation strategy and find your product.

Choose this if you use:	Surface coating or ligand on the magnetic beads	Target	Nonspecific binding	IP protocol time	Main benefits for IP	Thermo Scientific™ and Invitrogen™ products
Unconjugated primary antibody	**Protein A, G, A/G, or L**	Primary antibodies from most species; proteins A, G, and L bind different antibody species and subclasses with different specificities	Low	Dynabeads magnetic beads: <40 min Pierce beads: 130–180 min	• Dynabeads magnetic beads—fastest, easiest protocol with low nonspecific binding and high yield and reproducibility • Pierce Magnetic IP-MS Kit (Protein A/G) validated for mass spectrometry workflows • Pierce Crosslink kit includes DSS crosslinker	Dynabeads Protein A Dynabeads Protein A Immunoprecipitation Kit Dynabeads Protein G Dynabeads Protein G Immunoprecipitation Kit Pierce Classic Magnetic IP/Co-IP Kit Pierce Protein A/G Magnetic Beads Pierce Crosslink Magnetic IP/Co-IP Kit Pierce Magnetic IP-MS Kit (Protein A/G) Pierce Protein L Magnetic Beads
	Secondary antibodies	Mouse IgG or rabbit IgG	Low	Dynabeads magnetic beads: <40 min	• Fast and easy protocol • Low nonspecific binding • Specific binding of mouse or rabbit IgGs	Dynabeads M-280 Sheep Anti–Mouse IgG Dynabeads M-280 Sheep Anti–Rabbit IgG
	Epoxy- and NHS-activated beads	Any protein ligand (e.g., antibody, peptide)	Ultralow	Dynabeads magnetic beads: • Ab coupling time: overnight • Co-IP protocol time: 30–40 min Pierce beads: • Ab coupling time: 30– 60 min • Protocol time: 120 min	• Covalent coupling of the Ab gives ultralow nonspecific binding • No need for crosslinking • Gentle and efficient co-IP of even large protein complexes	Dynabeads Antibody Coupling Kit Dynabeads Co-Immunoprecipitation Kit Pierce NHS-activated magnetic beads
Biotinylated antibody	**Streptavidin**	Any biotinylated antibody or ligand	Low	Dynabeads magnetic beads: <40 min Pierce beads: 60–120 min	• Binds any biotinylated protein • For samples high in soluble IgGs • Recombinant Ab lacking the Fc region	Dynabeads MyOne Streptavidin T1 Dynabeads M-280 Streptavidin Pierce Streptavidin Magnetic Beads Pierce Magnetic IP-MS Kit (Streptavidin)
Recombinant protein	**Fusion tags**	Different beads bind proteins with the following tags: His, GST, DYKDDDDK (FLAG), HA, c-Myc	Low	Dynabeads His-tag beads: ~25 min Pierce beads: ~70 min	• Purify many different proteins incorporated with the same tag • No need for antibodies	Dynabeads His-Tag Isolation and Pulldown Pierce Anti-DYKDDDDK Magnetic Agarose Pierce HA-Tag Magnetic IP/Co-IP Kit Pierce c-Myc-Tag Magnetic IP/Co-IP Kit

Choose these products if you use unconjugated primary antibodies—your choice of antibody-binding products depends on your downstream application, or if you do not want the antibody co-eluted with your target protein.

Protein A, G, and A/G beads are most commonly used for IP and co-IP applications since unconjugated primary antibodies that face towards the protein target bind directly to the coated beads in a short and simple incubation step. Epoxy beads are often used to obtain ultralow nonspecific binding, or to avoid crosslinking, since the antibody is covalently coupled to the beads and not eluted with the target protein.

The Invitrogen™ epoxy beads and co-IP kit (including optimized buffers) are recommended for co-IP applications involving larger protein complexes.

Choose these products if you use biotinylated antibodies—your best choice when using a biotinylated antibody with streptavidin-coated beads for IP. These products are also useful in the following cases:

- If you have a sample rich in soluble IgGs
- If you have a recombinant antibody lacking Fc regions
- If you want to use streptavidin magnetic beads for pull-down applications using a biotinylated protein as bait

Choose these products if you have a recombinant protein with a fusion tag—the most popular fusion tags for recombinant protein expression are covered by Pierce and Dynabeads products. DYKDDDDK (FLAG epitope), HA-tag, and c-Myc tags are ideal for IP/co-IP applications, while His-tag and GST-tagged proteins are for pull-down assays.

Figures 5–8 show example data from experiments using various isolation strategies.

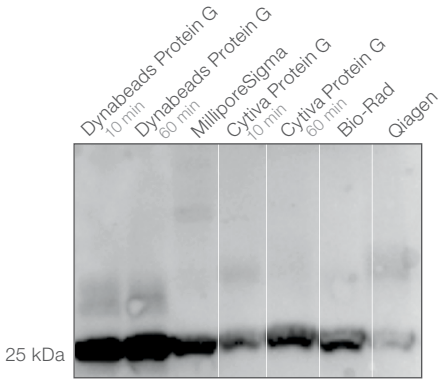


Figure 5. Protein yields determined by western blot analysis. Invitrogen™ Dynabeads™ Protein G magnetic beads have the best overall performance for yield, capacity, and nonspecific binding.

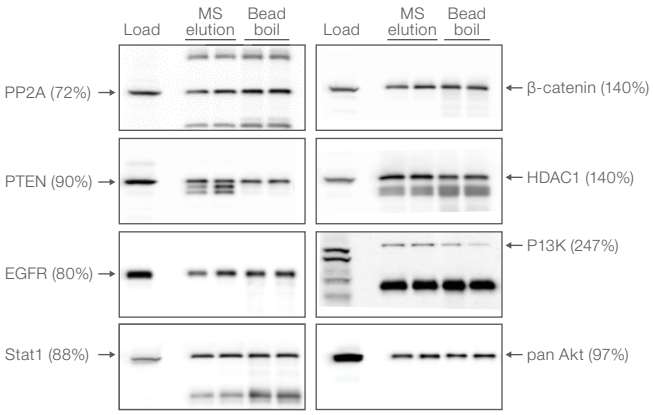


Figure 7. The Thermo Scientific™ Pierce™ MS-Compatible Magnetic IP Kit (streptavidin) allows for effective target capture and elution. Percentages indicate elution efficiency compared to bead boil. The eluates were analyzed by western blot. Antibodies were labeled with the Thermo Scientific™ Pierce™ Antibody Biotinylation Kit for IP and used with the Pierce MS-Compatible Magnetic IP Kit to immunoprecipitate target proteins from cell lysates.

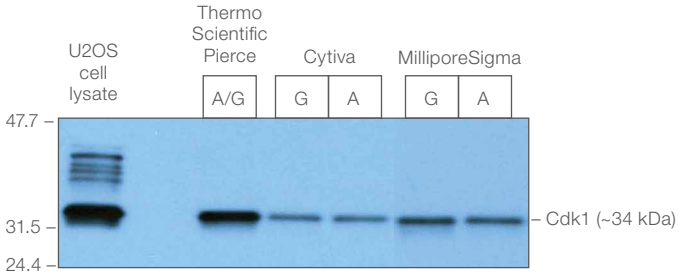


Figure 6. Higher IP yield with Protein A/G beads. U2OS (human osteosarcoma) cells were lysed in IP Lysis/Wash Buffer, and incubated with and without anti-Cdk1 antibody overnight at 4°C. Thermo Scientific™ Pierce™ Protein A/G Magnetic Beads were compared to Mag Sepharose™ Beads (Cytiva) and PureProteome™ Protein A and Protein G products (MilliporeSigma). The beads were washed multiple times using a KingFisher Flex instrument and then eluted with SDS-PAGE reducing sample buffer for 10 minutes at room temperature. The eluates were resolved by SDS-PAGE and analyzed by western blot for Cdk1.

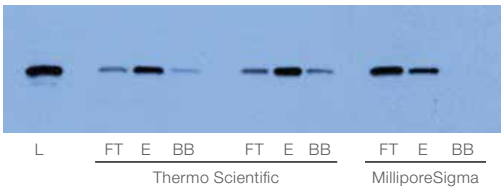


Figure 8. Comparison of protein purification using Thermo Scientific™ Pierce™ Anti-DYKDDDDK Affinity Resin and another supplier's product. C-terminal DYKDDDDK-tagged GFP protein was expressed using the Thermo Scientific™ 1-Step Human High-Yield Maxi *In Vitro* Translation (IVT) Kit and immunoprecipitated using Pierce Anti-DYKDDDDK Affinity Resin or Anti-FLAG™ M2 Affinity Gel (MilliporeSigma). Tagged proteins were competitively eluted with Pierce 3x DYKDDDDK Peptide and analyzed by western blot. Comparison of the starting lysate (L), flow-through (FT), eluate (E), and bead-boiled sample (BB) shows effective capture and elution of DYKDDDDK-tagged proteins.

To learn more about other magnetic bead products to help isolate everything from cells to exosomes, download the Dynabeads magnetic beads brochure at **thermofisher.com/dynabeads**

Magnetic stands

Multiple formats for low- to high-throughput sample processing

Invitrogen™ DynaMag™ magnets isolate any target in combination with Dynabeads magnetic beads. Reduce waiting time as these powerful magnets quickly pull the bead-bound target to the tube wall. The DynaMag magnets help to ensure optimal working positions and are functionally adapted to suit various workflows.

Highlights:

- **Optimized**—developed and certified for use with Dynabeads magnetic beads
- **Easy to handle**—designed with ergonomics in mind
- **More choices**—different formats to accommodate different volume and throughput needs

The Invitrogen™ DynaMag™-2 Magnet is our most commonly used magnet for immunoprecipitation and holds up to 16 standard 1.5–2 mL tubes in numbered spaces. The optimal working volume is 10–2,000 µL. There are eight positions on each side of the rack. The top rack can be quickly removed from the magnet in the base, ready for vortexing, rotation, or manual sample shaking. A center pin in the rack ensures equal vortexing of all tubes, providing excellent control and visibility during sample processing.

Invitrogen™ DynaMag™-Spin Magnet, which accommodates up to six (1.5 mL) microcentrifuge tubes, supports a working volume of 10–1,500 µL. The circular top rack can be quickly removed from the magnet in the base, ready for vortexing or manual sample shaking. Plate-based magnetic stands, such as the Invitrogen™ DynaMag™-96 series, are ideal for manual and automated work, with a footprint the same size as that of a 96-well plate. The recommended working volume is 5–200 µL.

DynaMag-2 Magnet

DynaMag-Spin Magnet

Sample rack for DynaMag-2 Magnet

DynaMag-96 Side Magnet
DynaMag-96 Bottom Magnet
DynaMag-96 Side Skirted Magnet

Test your protein research knowledge

Question: For large yields of highly purified protein, which purification strategy is the best?

A. Immunoprecipitation

B. Ion exchange chromatography

C. Affinity chromatography

D. Co-immunoprecipitation

Answer: C

Ordering information

Product	Quantity	Cat. No.
Purification		
Strong cation exchange purification resins		
POROS XS Resin	10 mL	82071
Strong anion exchange purification resins		
POROS XQ Resin	10 mL	82073
POROS HQ Resin	10 mL	82077
Antibody purification resins		
Pierce Protein A Plus Agarose	5 mL	22811
MabCaptureC High Capacity Protein A Resin	1 mL	A53031
Pierce High-Capacity Protein A MagBeads, alkali stable	1 mL	A53035
POROS MabCapture A Select Resin	15 mL	82080
Pierce Protein G Plus Agarose	2 mL	22851
POROS MabCapture G Select Resin	15 mL	82083
Pierce Protein A/G Magnetic Agarose Beads	1 mL	78609
Pierce Protein A/G Plus Agarose	2 mL	20423
POROS MabCapture A/G Select Resin	15 mL	82086
Pierce Protein L Agarose	2 mL	20510
Melon Gel Monoclonal IgG Purification Kit	1 kit	45214
Recombinant protein purification resins and magnetic beads		
Pierce High-Capacity Ni-IMAC Resin, EDTA compatible	1 mL	A50588
HisPur Ni-NTA Magnetic Beads	2 mL	88831
Pierce Ni-NTA Magnetic Agarose Beads	1 mL	78605
HisPur Ni-NTA Agarose Resin	10 mL	88221
HisPur Ni-NTA Superflow Agarose	10 mL	25214
HisPur Cobalt Agarose Resin	10 mL	89964
HisPur Cobalt Superflow Agarose	10 mL	25228
Pierce Glutathione Magnetic Agarose Beads	1 mL	78601
Pierce Glutathione Agarose	10 mL	16100
Pierce Glutathione Superflow Agarose	10 mL	25236
Pierce Anti-DYKDDDDK Affinity Resin	1 mL settled	A36801
Pierce Anti-c-Myc Agarose	2 mL	20168
Pierce Anti-HA Agarose	1 mL	26181
Biotin binding purification resins and magnetic beads		
Pierce Streptavidin Magnetic Beads	1 mL	88816
Pierce High Capacity Streptavidin Agarose	2 mL	20357
Pierce High Capacity NeutrAvidin Agarose	5 mL	29202
Pierce Monomeric Avidin Agarose	5 mL	20228
Activated support resins and magnetic beads		
Pierce NHS-Activated Agarose, Dry	1 g	26196
AminoLink Plus Coupling Resin	10 mL	20501
SulfoLink Coupling Resin	10 mL	20401
CarboxyLink Coupling Resin	25 mL	20266
GlycoLink Immobilization Kit	10 columns	88941
Pierce NHS-Activated Magnetic Beads	1 mL	88826
Dynabeads M-270 Epoxy	60 mg	14301
Dynabeads MyOne Tosylactivated	2 mL	65501
Dynabeads M-270 Amine	2 mL	14307D

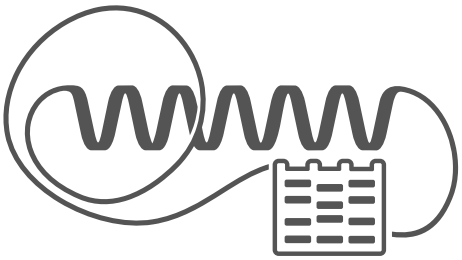
To view additional pack sizes and products, go to **thermofisher.com/proteinpurification**

Product	Quantity	Cat. No.
Immunoprecipitation		
Immunoprecipitation using magnetic beads		
Dynabeads Protein A	1 mL	10001D
Dynabeads Protein A Immunoprecipitation Kit	2 mL	10006D
Dynabeads Protein A IP Kit and Magnet Starter Pack	40 reactions	10018D
Dynabeads Protein A and Magnet Starter Pack	40 reactions	10013D
Dynabeads Protein G	1 mL	10003D
Dynabeads Protein G Immunoprecipitation Kit	40 reactions	10007D
Dynabeads Protein G IP Kit and Magnet Starter Pack	40 reactions	10019D
Dynabeads Protein G and Magnet Starter Pack	40 reactions	10014D
Dynabeads Protein A/Protein G and Magnet Starter Pack	40 reactions	10015D
Pierce Protein A/G Magnetic Beads	1 mL	88802
Pierce Classic Magnetic IP/Co-IP Kit	40 reactions	88804
Pierce Crosslink Magnetic IP/Co-IP Kit	40 reactions	88805
Pierce MS-Compatible Magnetic IP Kit (Protein A/G)	40 reactions	90409
Pierce Protein L Magnetic Beads	1 mL	88849
Dynabeads M-280 Sheep Anti-Mouse IgG	2 mL	11201D
Dynabeads M-280 Sheep Anti-Rabbit IgG	2 mL	11203D
Dynabeads Antibody Coupling Kit	1 kit	14311D
Dynabeads Co-Immunoprecipitation Kit	40 reactions	14321D
Pierce Direct Magnetic IP/Co-IP Kit	40 reactions	88828
Dynabeads M-280 Streptavidin	2 mL	60210
Dynabeads MyOne Streptavidin C1	2 mL	65001
Pierce MS-Compatible Magnetic IP Kit (Streptavidin)	40 reactions	90408
Dynabeads His-Tag Isolation and Pulldown	2 mL	10103D
Pierce Anti-DYKDDDDK Magnetic Agarose	1 mL	A36797
Pierce HA-Tag Magnetic IP/Co-IP Kit	40 reactions	88838
Pierce c-Myc-Tag Magnetic IP/Co-IP Kit	40 reactions	88844
Immunoprecipitation kits using agarose resin		
Pierce Classic IP Kit	50 reactions	26146
Pierce Crosslink IP Kit	50 reactions	26147
Pierce Direct IP Kit	50 reactions	26148
Pierce Co-Immunoprecipitation Kit	50 reactions	26149
GlycoLink IP Kit	25 reactions	88943
Pierce Biotinylated Protein Interaction Pull-Down Kit	25 reactions	21115
EZ-Link Desthiobiotinylation and Pull-Down Kit	5 reactions	16138
Pierce c-Myc-Tag IP/Co-IP Kit	25 reactions	23620
Pierce HA-Tag IP/Co-IP Kit	25 reactions	26180
Pierce GST Protein Interaction Pull-Down Kit	25 reactions	21516
Pierce His Protein Interaction Pull-Down Kit	25 reactions	21277

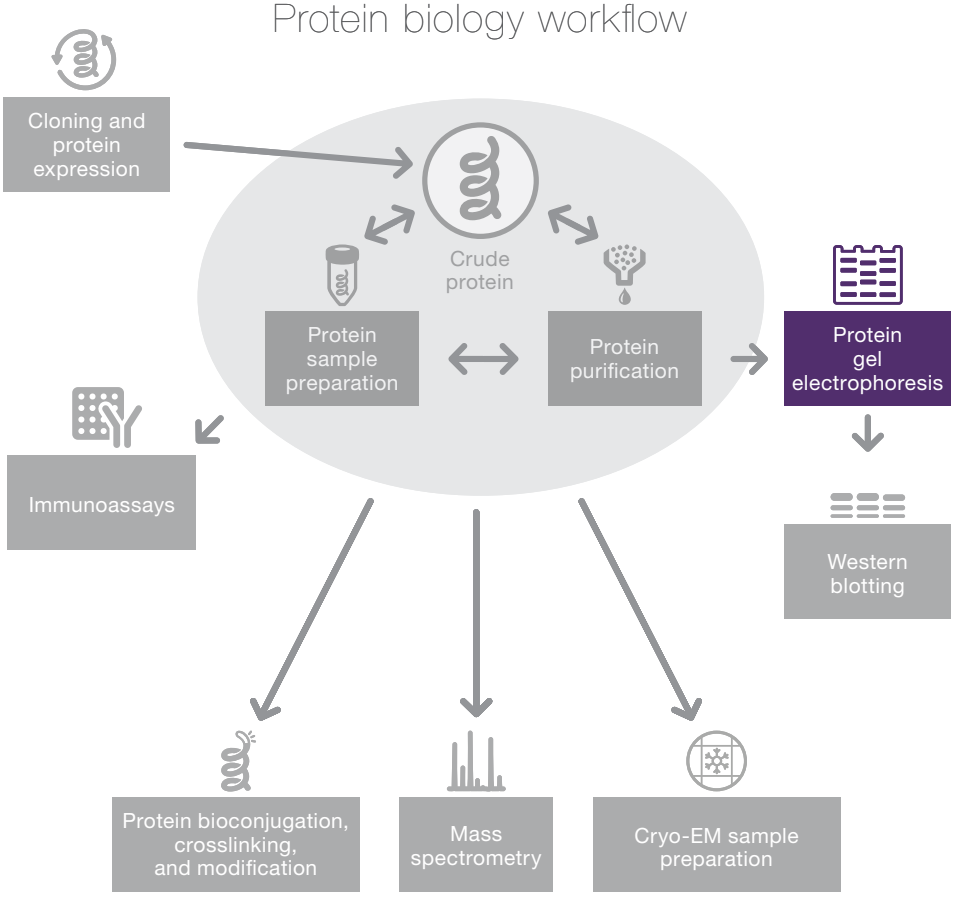
To view additional pack sizes and products, go to **thermofisher.com/immunoprecipitation**

Protein gel electrophoresis

Gel electrophoresis is a technique in which charged molecules are separated according to physical properties such as charge or mass as they are forced through a sieving gel matrix by an electrical current. Proteins are commonly separated in this manner using polyacrylamide gel electrophoresis (PAGE) to identify individual proteins in complex samples or to examine multiple proteins within a single sample.



Protein biology workflow



Several forms of PAGE exist, each providing different types of information about the proteins under investigation. Nondenaturing PAGE, also called native PAGE, separates proteins based on their charge and size, preserving their conformation and activity. Denaturing and reducing SDS-PAGE, the most widely used electrophoresis technique, separates proteins by mass. Two-dimensional (2D) PAGE separates proteins by isoelectric point in the first dimension and by mass in the second dimension.

SDS-PAGE separates proteins primarily by mass. The ionic detergent sodium dodecyl sulfate (SDS) binds to the proteins in the mixture shielding their respective charge and making them all uniformly negatively charged. Thus, when a current is applied, all SDS-bound proteins in a sample will migrate through the gel toward the positively charged electrode. Proteins with less mass travel more quickly through the gel than those with greater mass because of the sieving effect of the gel matrix.

Once separated by electrophoresis, proteins can be detected in a gel with various stains, transferred onto a membrane for detection by western blotting, or excised and extracted for analysis by mass spectrometry. Protein gel electrophoresis is, therefore, a common step in many kinds of proteomic analyses.

Gel matrix

Acrylamide is the base component used in electrophoretic gels to separate proteins by size; when mixed with bisacrylamide in the presence of ammonium persulfate, it forms a crosslinked polymer network. Ammonium persulfate acts as a polymerizing agent by producing free radicals, while TEMED (N,N,N′,N′-tetramethyl ethylenediamine) is added to catalyze and speed up the reaction. The size of the pores created in the gel is inversely related to the amount of acrylamide and the bisacrylamide to acrylamide ratio.

Electrophoresis gels are formulated in buffers that conduct an electrical current through the matrix. The solution is poured into the thin space between two glass or plastic plates of an assembly called a cassette. Once the gel polymerizes, the cassette is mounted into an apparatus so that opposite edges (top and bottom) are placed in contact with buffer chambers containing a cathode and anode, respectively. When proteins are added in wells at the top edge and current is applied, the proteins are drawn by the current through the matrix slab and separated by its sieving properties.

To obtain optimal resolution of proteins, a stacking gel is cast over the top of the resolving gel. The stacking gel has a lower concentration of acrylamide, lower pH, and a different ionic content. This allows the proteins in a loaded sample to be concentrated into a tight band during the first few minutes of electrophoresis before entering the resolving portion of a gel. A stacking gel is not necessary when using a gradient gel, as the gradient itself performs this function.

Linear vs. gradient gels

Gels that have a single acrylamide percentage are referred to as linear gels, and those with a range are referred to as gradient gels. The advantage of using a gradient gel is that it allows the separation of a broader range of proteins than does a linear gel.

Mini vs. midi protein gels

Commercial gels are available in two size formats: mini gels and midi gels. Both gels have similar run lengths, but midi gels are wider than mini gels, allowing midi gels to have more wells or larger wells. The additional wells in the midi gels permit more samples or large sample volumes to be loaded onto one gel.

Homemade vs. precast gels

Traditionally, researchers poured their own gels using standard recipes that are widely available in protein method books. Most laboratories now depend on the convenience and consistency afforded by commercially available ready-to-use precast gels. Precast gels are available in a variety of percentages; these include difficult-to-pour gradient gels that provide excellent resolution and separate proteins over the widest possible range of molecular weights. Technological innovations in buffer and gel polymerization methods enable manufacturers to produce gels with greater uniformity and longer shelf life than with traditional equipment and methods. In addition, precast polyacrylamide gels obviate the need to work with the acrylamide monomer—a known neurotoxin and suspected carcinogen.

Protein standards

To assess the relative molecular weight (MW) of a protein on a gel, protein MW markers are run in the outer lanes of the gel. A standard curve can be constructed using the distances migrated by each marker protein. The distance migrated by the unknown protein is then plotted, and the molecular weight is interpolated from the standard curve.

Several kinds of ready-to-use protein MW markers are available that are labeled or prestained for different modes of detection. These are pre-reduced and, therefore, primarily suited for SDS-PAGE rather than native PAGE. MW markers are detectable via their specialized labels or by ordinary protein staining methods.

Overview of precast gels

Invitrogen™ precast gels offer convenience, speed, and consistency. We offer precast gels in four different formulations and a wide variety of percentages, gradients, and sample well configurations. The choice of whether to use one formulation or another depends on the abundance of the protein you are separating, the size of the protein, and your downstream application.

Four gel options to fit your protein separation needs:

- Bis-Tris gels for broad-range, low-abundance protein separation, or for downstream applications requiring high protein integrity, such as posttranslational modification analysis, mass spectrometry, or sequencing
- Tris-glycine gels for broad-range, high-abundance protein separation
- Tris-acetate gels for high molecular weight protein separation, up to 500 kDa
- Tricine gels for low molecular weight protein and peptide separation

Find out more at **thermofisher.com/proteingels**

Protein gel performance guarantee

We stand behind the quality of our high-performance protein gels. Purchase Invitrogen™ protein gels with confidence, knowing that our gels are backed by our protein gel performance guarantee. If an Invitrogen protein gel does not perform in your experiment as described on our website or Certificate of Analysis, we will replace the product at no cost to you, or we will provide you with a credit for future purchase.

Learn more at **thermofisher.com/proteingelguarantee**

Protein gel welcome packs

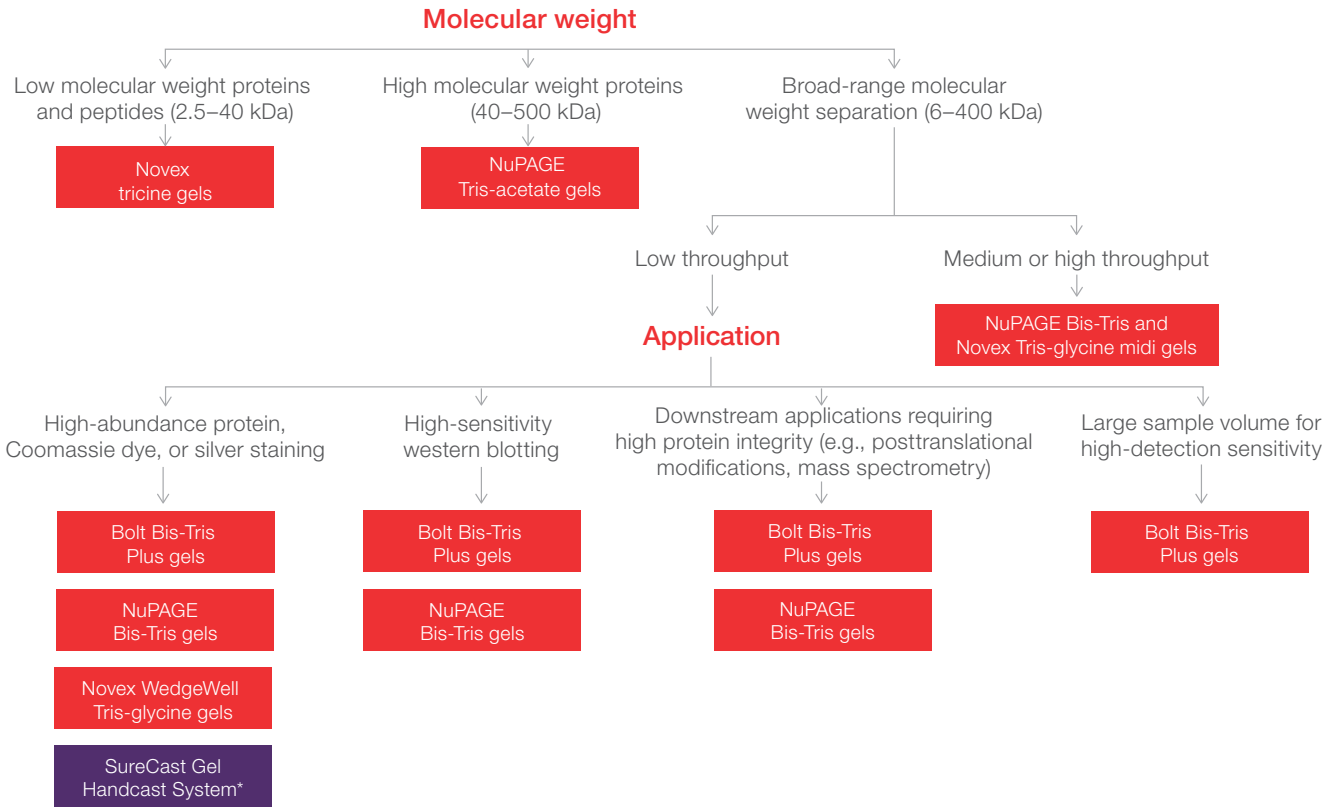
Invitrogen™ protein gel welcome packs contain the components for outstanding protein separation and are available for mini- and midi-format protein gels. The typical protein gel welcome pack provides all of the necessary gels, buffers, and reagents you need, as well as an Invitrogen™ Mini Gel Tank, Invitrogen™ XCell SureLock™ Mini-Cell, or Invitrogen™ SureLock™ Tandem Midi Gel Tank. Protein gel welcome packs are also available in mini gel and midi gel formats designed for use in protein expression experiments and subsequent purification steps.

Find out more at **thermofisher.com/proteingelwelcome**

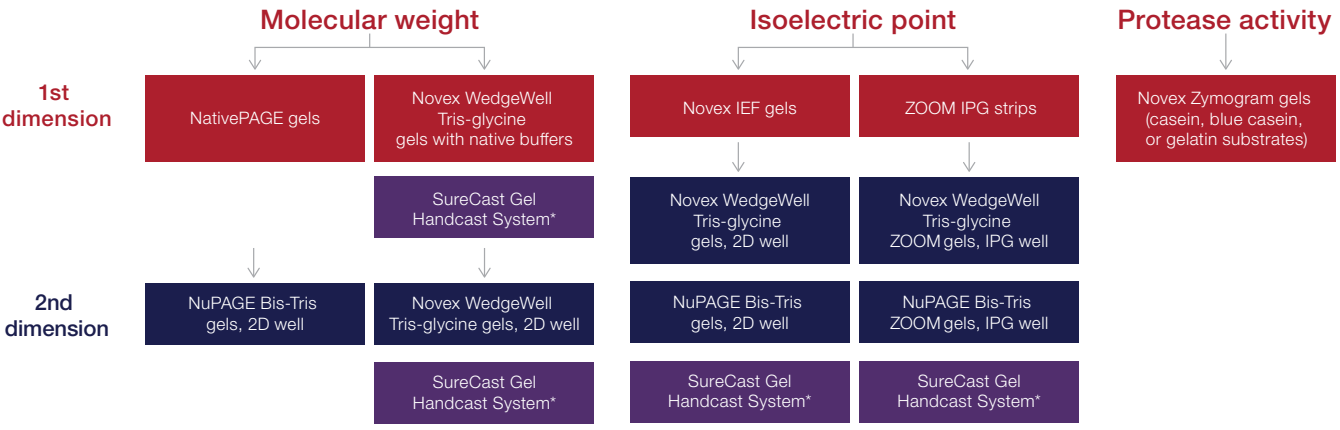
Gel selection guide

Find the right gel for your research needs based on molecular weight, downstream applications, and throughput requirements.

Denaturing separation



Native separation



* Pour your own Tris-glycine gels with this system.

Find the right gel using our interactive gel selection tool at **thermofisher.com/proteingelguide**

SureCast Gel Handcast Station

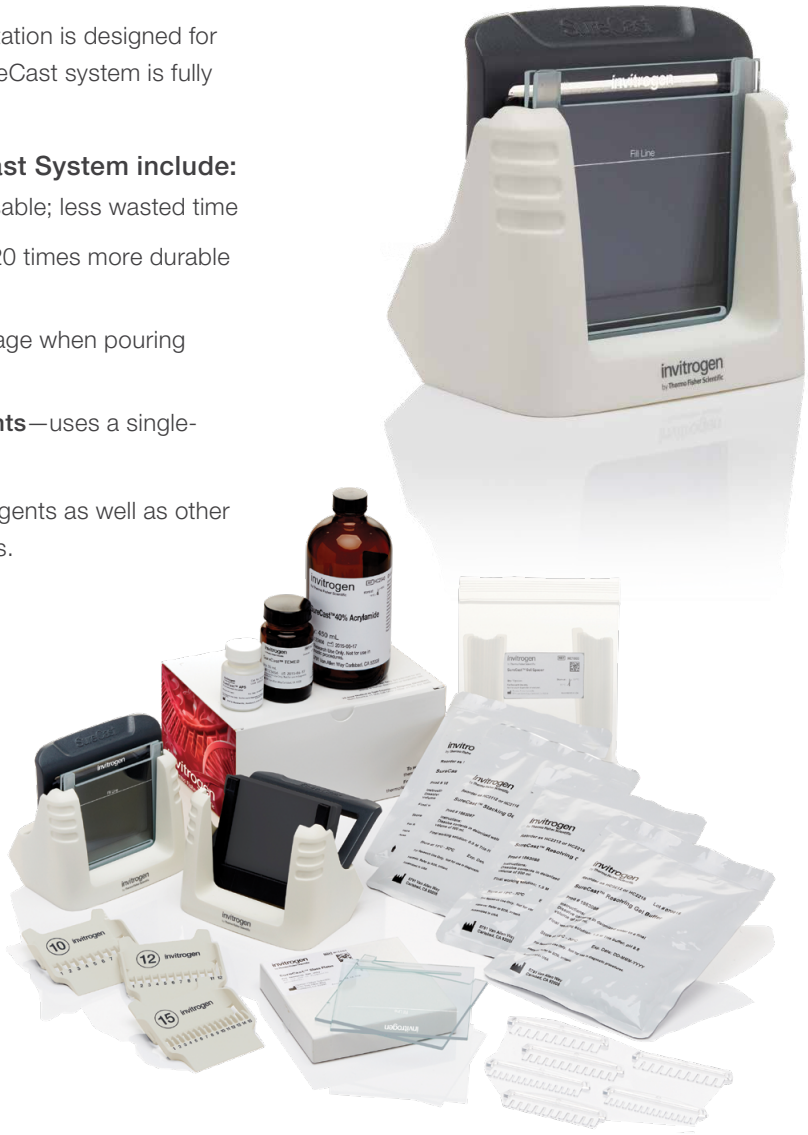
Pour your own gels

The Invitrogen™ SureCast™ Gel Handcast Station is designed for 100% leak-free protein gel casting. The SureCast system is fully compatible with our Mini Gel Tank.

Benefits of the SureCast Gel Handcast System include:

- **Leak-free design**—gels that are more usable; less wasted time
- **Superior glass plate durability**—up to 20 times more durable than other suppliers' plates
- **Unique tilt feature**—helps minimize spillage when pouring acrylamide solutions
- **Simple assembly of casting components**—uses a single-motion, load-and-lock mechanism

Use Invitrogen™ SureCast™ handcasting reagents as well as other popular polyacrylamide gel casting reagents.



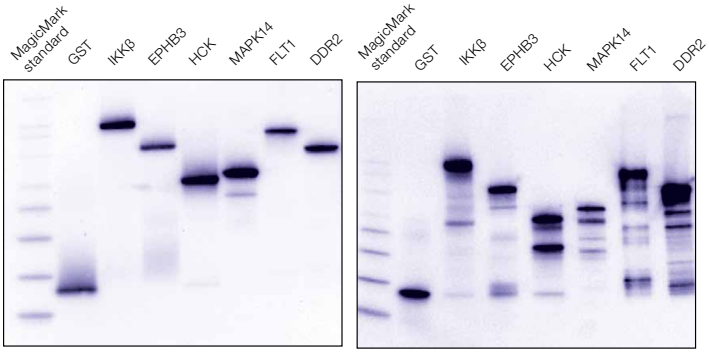
Bolt Bis-Tris Plus and NuPAGE Bis-Tris mini gels

Neutral-pH gel systems for optimal separation

Invitrogen™ Bolt™ Bis-Tris Plus and NuPAGE™ Bis-Tris gels are precast polyacrylamide gels designed for optimal separation of a broad molecular weight range of proteins under denaturing conditions during gel electrophoresis. These gels help deliver consistent performance with a neutral-pH environment to minimize protein degradation, resulting in sharper bands without typical gel "smiling". Additionally, preserving protein integrity becomes particularly important when separating low-abundance proteins. The unique wedge-well design of Bolt Bis-Tris Plus gels allows loading up to twice the sample volume of other precast gels. In addition, Bolt gels can be run in as little as 20 minutes. Bolt Bis-Tris Plus and NuPAGE Bis-Tris gels are ideal for western blot transfer and analysis along with any other technique where protein integrity is crucial. Bolt Bis-Tris Plus gels are available in the mini-gel format and NuPAGE Bis-Tris gels are available in mini- and midi-gel formats, as well as multiple thicknesses.

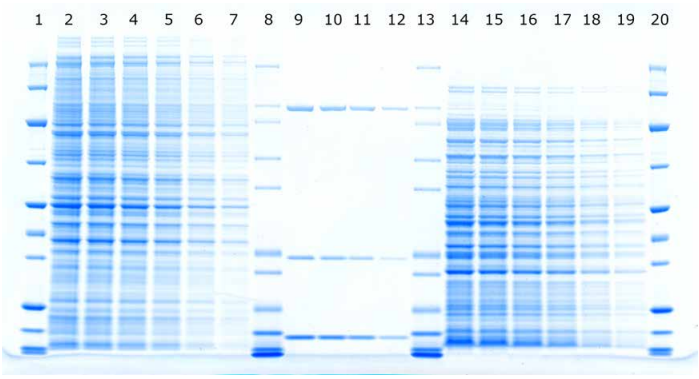
Bolt Bis-Tris Plus and NuPAGE Bis-Tris gels offer:

- **Preserved protein integrity**—neutral-pH formulation minimizes protein modifications and degradation (Figure 1)
- **Superior band quality and band volume**—Bis-Tris gels are designed to deliver sharp, straight bands with higher band volume (Figure 2)
- **High sample volume capacity**—wedge-well design of Bolt Bis-Tris Plus mini gels allows detection of proteins in very dilute samples or measurement of low-abundance proteins
- **More efficient western blot transfer**—neutral pH prevents reoxidation of reduced samples during protein transfer
- **High lot-to-lot consistency**—coefficient of variation (CV) of only 2% for R_f values (migration)
- **Long shelf life**—16 months at room temperature



Bolt Bis-Tris Plus gel | Bio-Rad TGX Tris-glycine gel

Figure 1. Bolt Bis-Tris Plus mini gels help provide better western blotting results. A western blot of a Bolt gel shows clean, sharp protein signals corresponding to only full-length proteins, whereas a western blot of a Bio-Rad™ TGX™ gel shows multiple low molecular weight degradation products. Protein kinases implicated in cancer (IKKβ, EPHB3, HCK, MAPK14, FLT1, and DDR2) were analyzed on a Bolt Bis-Tris Plus gel and a Bio-Rad TGX Tris-glycine gel. The purified kinases (50 ng each), along with Invitrogen™ MagicMark™ XP Western Protein Standard and purified recombinant GST protein, were loaded on a 10-well, 4–12% Bolt gel and a 10-well, 4–20% Bio-Rad TGX gel. The samples were separated and transferred to 0.45 µm PVDF membranes using the respective manufacturers' protocols. Immunodetection was performed using an anti-GST antibody and Invitrogen™ WesternBreeze™ chemiluminescence detection. The blots were imaged using an LAS-1000 system (FujiFilm).



NuPAGE Bis-Tris 4–12% gradient midi gel

Figure 2. Publication-quality protein electrophoresis gel results using a NuPAGE Bis-Tris 4–12% gradient midi gel and the SureLock Tandem Midi Gel Tank. Lanes 1, 20: 5 µL Thermo Scientific™ PageRuler™ Broad Range Unstained Protein Ladder; lanes 2–7: 10 µg, 8 µg, 6 µg, 4 µg, 2 µg, 1 µg of HeLa lysate; lanes 8, 13: 5 µL Invitrogen™ Novex™ Mark12™ Unstained Standard; lanes 9–12: 240 ng, 180 ng, 120 ng, 60 ng of protein mix containing β-galactosidase, lactate dehydrogenase, and lysozyme; lanes 14–19: 10 µg, 8 µg, 6 µg, 4 µg, 2 µg, 1 µg of *E. coli* lysate. The gel was run in Invitrogen™ NuPAGE™ MOPS running buffer, and stained with Invitrogen™ SimplyBlue™ SafeStain.

Find out more at **thermofisher.com/nupage**

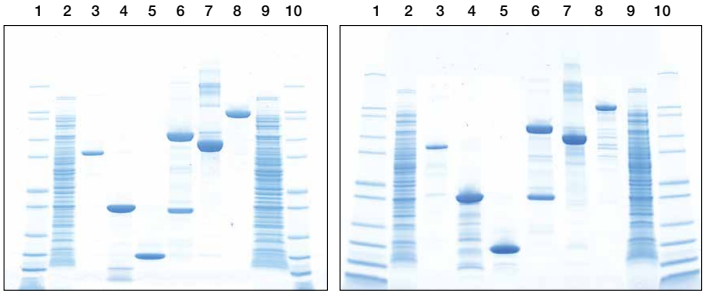
Novex Tris-glycine gels

Load up to 60 µL of sample

The Invitrogen™ Novex™ Tris-Glycine mini gels, WedgeWell™ format, and Invitrogen™ Novex™ Tris-Glycine Plus midi gels are polyacrylamide gels based on traditional Laemmli chemistry that enable the use of Laemmli sample and running buffers. Novex Tris-Glycine gels provide high-quality performance and separation of a wide range of proteins into well-resolved bands.

Highlights:

- **Wedge-shaped wells**—easily load up to 60 µL of sample without sacrificing gel width or length (mini gel only)
- **High performance**—excellent protein band resolution and sharpness (Figure 3)
- **Fast run conditions**—quickly separate your proteins using constant voltage in less than 60 minutes
- **Flexible**—Novex Tris-glycine gels do not contain SDS and can be used to run proteins in native or in denatured form



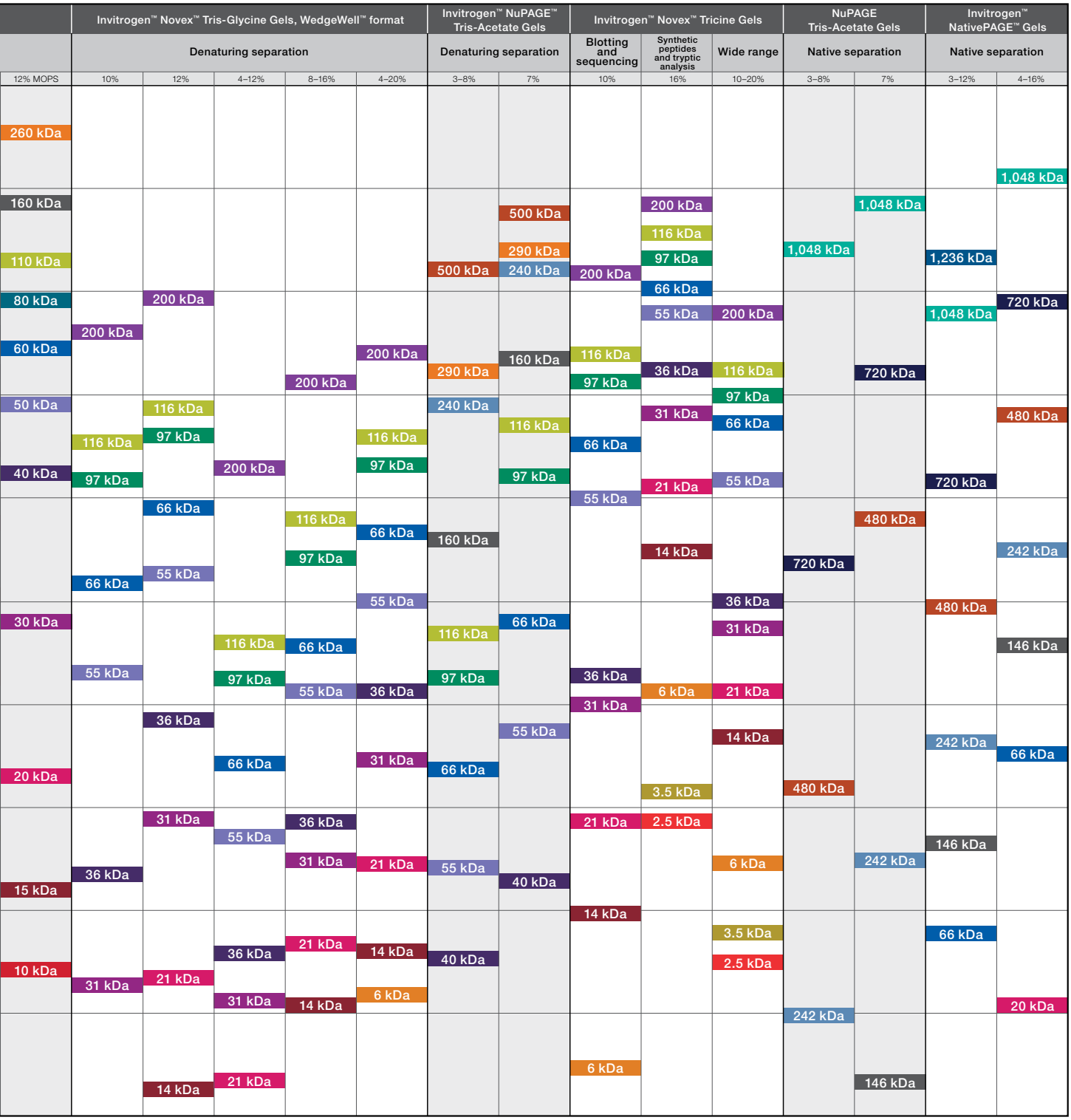
Novex 4–20% Tris-glycine mini gel, WedgeWell format | **Bio-Rad TGX 4–20% gel**

Figure 3. Better protein resolution and band sharpness with Novex Tris-glycine mini gels, WedgeWell format. Protein ladder, purified proteins, and *E. coli* lysate were loaded on a gradient Novex 4–20% Tris-Glycine mini gel, WedgeWell format, and a Bio-Rad TGX 4–20% gradient gel. The Bio-Rad TGX gel displays numerous low molecular weight protein degradation products below major bands in lanes 3, 4, 7, and 8 that are not seen in the Novex Tris-glycine gel. The Novex gel also displays better protein band sharpness and resolution of lysate than the Bio-Rad gel. Lanes 1, 10: 5 µL Invitrogen™ Mark12™ Unstained Standard; lane 2: 10 µg *E. coli* lysate (10 µL); lane 3: 6 µg catalase (10 µL); lane 4: 6 µg carbonic anhydrase (10 µL); lane 5: 6 µg lysozyme (10 µL); lane 6: 6 µg hIgM (10 µL); lane 7: 6 µg BSA (10 µL); lane 8: 6 µg β-galactosidase (10 µL); lane 9: 20 µg *E. coli* lysate (20 µL).

Find out more at **thermofisher.com/novexwedge**

Protein mini gel migration chart

Use this chart to compare migration patterns of proteins of various molecular weights across the Invitrogen™ precast gel product line.


Invitrogen™ Novex™ Tris-Glycine Gels, WedgeWell™ format
Invitrogen™ NuPAGE™ Tris-Acetate Gels
Invitrogen™ Novex™ Tricine Gels
NuPAGE Tris-Acetate Gels
Invitrogen™ NativePAGE™ Gels
Denaturing separation
Denaturing separation
Blotting and sequencing
Synthetic peptides and tryptic analysis
Wide range
Native separation
Native separation
12% MOPS
10%
12%
4–12%
8–16%
4–20%
3–8%
7%
10%
16%
10–20%
3–8%
7%
3–12%
4–16%
260 kDa
160 kDa
110 kDa
80 kDa
60 kDa
50 kDa
40 kDa
30 kDa
20 kDa
15 kDa
10 kDa
500 kDa
290 kDa
240 kDa
200 kDa
116 kDa
97 kDa
66 kDa
55 kDa
36 kDa
31 kDa
21 kDa
14 kDa
6 kDa
3.5 kDa
2.5 kDa
1,048 kDa
720 kDa
480 kDa
242 kDa
146 kDa

Electrophoresis chambers

Ideal for multiple applications and needs

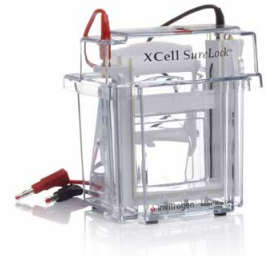
The Invitrogen™ gel portfolio offers four gel electrophoresis chamber systems to help you achieve your application and throughput needs (Table 1). The Invitrogen™ Mini Gel Tank is designed for more intuitive use and greater convenience compared to traditional electrophoresis tanks. The unique side-by-side tank design allows you to perform electrophoresis of 1 or 2 mini gels, and less running buffer is required because the gel chambers are separated. The Invitrogen™ XCell4 *SureLock*™ Midi-Cell Electrophoresis System is ideal for higher throughput experiments. The user-friendly design allows electrophoresis of up to 4 midi gels simultaneously.

Highlights:

- **Flexible**—run 1–4 gels simultaneously, depending on the chamber
- **Optimized**—chambers amenable to wet transfer or midi-gel applications
- **Unique**—Mini Gel Tank enables side-by-side gel loading and enhanced viewing during use

Watch our Mini Gel Tank video at **thermofisher.com/minigeltank**

Table 1. Electrophoresis chamber systems.

	Mini Gel Tank	XCell *SureLock* Mini-Cell	SureLock Tandem Midi Gel Tank	XCell4 *SureLock* Midi-Cell
				
Gel capacity	Up to 2 mini gels	Up to 2 mini gels	Up to 2 midi gels	Up to 4 midi gels
Cell dimensions (L x W x H; height with lid on)	32 x 11.5 x 16 cm	14 x 13 x 16 cm	25 x 17.9 x 17.3 cm	21 x 19 x 16 cm
Advantages	• The Mini Gel Tank is versatile and compatible with all Invitrogen precast and handcast mini gels; the unique tank design enables convenient side-by-side gel loading and enhanced viewing during use • Mini Blot Module is available for wet protein transfers	• Instrument incorporates a gel tension wedge in place of the rear wedge used on earlier models • XCell II Blot Module is available for wet protein transfers	• Easy-to-use apparatus, with separate chambers for each gel, enabling scalable buffer usage • SureLock Tandem Midi Blot Module is available for wet protein transfers	• Advanced apparatus for easier, more reliable electrophoresis with midi gels

Find out more at **thermofisher.com/electrophoresischambers**

PowerEase Touch Power Supplies

Easy touchscreen programming and operation

The Invitrogen™ PowerEase™ Touch Power Supplies make setting up custom protocols or selecting one of the several preprogrammed gel electrophoresis and transfer methods a breeze with an improved 4.3-inch backlit LCD touchscreen display and user interface. The power supplies are ideal for DNA or RNA electrophoresis, SDS-PAGE, and native PAGE. The PowerEase Touch Power Supplies offer four sets of output jacks that can be used simultaneously and three modes: constant voltage, constant current, and constant power for flexibility of use and efficiency. The sturdy polyurethane feet and stackable housing design allow stacking of power supplies for a reduced footprint on the lab bench.

- **Ease of use**—LCD touchscreen display and user interface show clear menu prompts for easy use by hand or stylus and convenient monitoring of run progress
- **Convenient**—four sets of output terminals allow running of multiple electrophoresis units
- **Customizable**—program up to 100 custom methods, 20 steps per method, 999 minutes per step, or select one of several preprogrammed Invitrogen gel electrophoresis and transfer methods
- **Safety**—features automatic No Load, Over Temperature, Over Voltage, Over Current, Load Change, and Ground Leak detection

The PowerEase Touch 120W Power Supply is a medium-throughput power supply, with a maximum output of 300 V, 500 mA, and 120 W. The PowerEase Touch 350W Power Supply is a high-throughput power supply, with a maximum output of 300 V, 3 A (3,000 mA), and 350 W.

Protein ladders and standards

Prestained and unstained formats with exceptional lot-to-lot consistency

We offer a broad range of prestained and unstained protein ladders in a ready-to-use format to facilitate easy protein analysis during gel electrophoresis and western blotting (Tables 2 and 3).

Highlights:

- **Performance**—sharp protein bands and consistent migration patterns provide easy molecular weight determination (Figure 4)
- **Convenience**—protein ladders are ready to load, with no heating required
- **Reliability**—exceptional lot-to-lot consistency and reproducibility

Table 2. Prestained and unstained protein ladders.

Prestained protein ladders	
Low range	Spectra Multicolor Low Range Protein Ladder
Broad range	PageRuler Plus Prestained Protein Ladder
High range	HiMark Prestained Protein Standard

Recommended for:

- Determining approximate molecular weight
- Monitoring the progress of electrophoresis runs
- Estimating the efficiency of protein transfer to the membrane during western blotting

Unstained protein ladders	
Low range	PageRuler Unstained Low Range Protein Ladder
Broad range	PageRuler Unstained Broad Range Protein Ladder
High range	HiMark Unstained Protein Standard

Recommended for:

- Determining precise molecular weight of target protein

Other	
Western blot	MagicMark XP Western Protein Standard
Specialty	iBright Prestained Protein Ladder PageRuler Prestained NIR Protein Ladder BenchMark Fluorescent Protein Standard BenchMark His-tagged Protein Standard IEF Marker 3–10

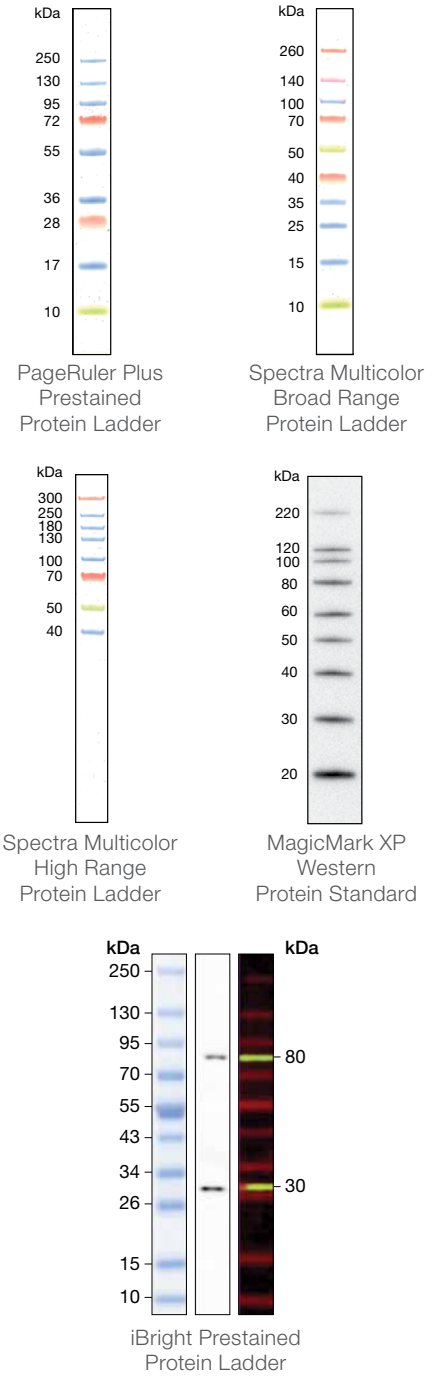


Figure 4. Migration patterns of protein ladders. Thermo Scientific™ PageRuler™ and Spectra™ ladders are visualized directly after gel separation. The Invitrogen™ MagicMark™ XP standard is visualized by chemiluminescent substrate detection. The Invitrogen™ iBright™ Prestained Protein Ladder has three methods of detection. Left: prestained ladder visualized after gel separation; center: chemiluminescent substrate detection of western blot; right: near-IR fluorescence detection of western blot.

Find out more at **thermofisher.com/proteinladders**

Table 3. Selection guide for protein ladders and standards.

Category	Product	Advantages	Range	No. of bands	Recommended for
Unstained ladders and standard					
Unstained standards	PageRuler Unstained Low Range Protein Ladder	Sharp bands and precise molecular weight estimation for low molecular weight proteins	3.4–100 kDa	8	MW determination, gel staining, chemiluminescent band visualization
	PageRuler Unstained Broad Range Protein Ladder	Sharp bands and precise molecular weight estimation for a wide range of proteins	5–250 kDa	14	MW determination, gel staining, chemiluminescent band visualization
	HiMark Unstained Protein Standard	Convenient molecular weight estimation for high molecular weight proteins	20–1,200 kDa	8	MW determination (native gels), gel staining, chemiluminescent band visualization
Prestained protein ladders and standard					
Prestained standards	iBright Prestained Protein Ladder	• **Fluorescence visualization**—detect the ten stained proteins using the 670 nm red laser or 700 nm channel of a fluorescence imager • **Western blot confirmation**—detect the two unstained proteins using the detection system for the target protein • **Reference band**—55 kDa band has greater intensity for easy orientation	11–250 kDa	12	Western blotting: detection of the two unstained bands using the detection method for the target protein; compatible with chemiluminescent substrates and fluorescent secondary antibodies
	PageRuler Prestained Protein Ladder	Outstanding clarity for easy molecular weight determination of low molecular weight proteins	10–180 kDa	10	MW determination, protein band visualization, monitoring electrophoresis run and transfer, chemiluminescent band visualization
	PageRuler Plus Prestained Protein Ladder	Outstanding clarity for easy molecular weight determination of a broad range of proteins	10–250 kDa	9	MW determination, protein band visualization, monitoring electrophoresis run and protein transfer
	HiMark Prestained Protein Standard	Superb analysis of high molecular weight proteins	30–460 kDa	9	MW determination (high MW proteins), protein band visualization, monitoring electrophoresis run and protein transfer
	Spectra Multicolor Broad Range Protein Ladder	Superior visualization and analysis of a broad range of proteins	10–260 kDa	10	MW determination, protein band visualization (best), monitoring electrophoresis run and protein transfer
	Spectra Multicolor High Range Protein Ladder	Superior and convenient visualization of high molecular weight proteins	40–300 kDa	8	MW determination, protein band visualization (best), monitoring electrophoresis run and protein transfer
Other ladders and standards					
Chemiluminescent standard	MagicMark XP Western Protein Standard	Accurate molecular weight estimation directly on western blots	20–220 kDa	9	MW determination, gel staining, chemiluminescent band visualization (best)
Near-infrared (NIR) standard	PageRuler Prestained NIR Protein Ladder	Sharp prestained standard for near-IR fluorescent visualization and protein sizing	11–250 kDa	10	MW determination
Fluorescent standard	BenchMark Fluorescent Protein Standard	Efficient estimation of molecular weight by fluorescent detection	11–155 kDa	7	MW determination, chemiluminescent band visualization
His-tag standard	BenchMark His-tagged Protein Standard	Convenient detection and protein sizing of His-tagged proteins	10–160 kDa	10	MW determination, gel staining, chemiluminescent band visualization (using anti-6xHis antibody)
IEF	IEF Marker 3–10	Accurate determination of protein isoelectric points	pI 3.5–10.7	13	pI determination

Protein stains

Optimized formulations for specific applications

Once protein bands have been separated by electrophoresis, they can be directly visualized using different methods of in-gel detection. Over the past several decades, demands for improved sensitivity for small sample sizes and compatibility with downstream applications and detection instrumentation have driven the development of several basic staining methods. Each method has particular advantages and disadvantages, and a number of specific formulations of each type of method provide optimal performance for various situations. Our portfolio includes Coomassie, silver, fluorescent, and specialty gel stains (Table 4).

Highlights:

- **Convenient**—most formulations are ready to use
- **Optimized**—reagents and kits developed for specific applications and workflows
- **Flexible offering**—multiple options to meet sensitivity or budget needs

Table 4. Gel stain selection guide.

Protein staining			
	Coomassie staining	Silver staining	Fluorescent protein staining
Sensitivity	25 ng	0.5 ng	0.5 ng
Ease of use	+++	+	+
Mode of action	In acidic buffer conditions, Coomassie stain binds to basic and hydrophobic residues of proteins, changing from dull reddish-brown to intense blue.	Silver ions interact and bind with carboxylic acid groups (Asp and Glu), imidazole (His), sulfhydryls (Cys), and amines (Lys). Silver ions are reduced to metallic silver, resulting in a brown-black color.	Most fluorescent stains involve simple dye-binding mechanisms rather than chemical reactions that alter protein functional groups.
Detection	Visual	Visual	Compatible imaging system
Compatibility with downstream applications	Compatible with mass spectrometry (MS) and sequencing	Certain formulations are compatible with MS	Most stains are compatible with MS
Products	**Value:** PageBlue Protein Staining Solution **Performance:** SimplyBlue SafeStain **Premium:** Imperial Protein Stain	**Value:** Pierce Silver Stain Kit **Performance:** SilverXpress Silver Staining Kit **Mass spectrometry:** Pierce Silver Stain for Mass Spectrometry	**Value:** SYPRO Red Protein Gel Stain **Performance:** SYPRO Orange Protein Gel Stain **Premium:** SYPRO Ruby Protein Gel Stain

Test your protein research knowledge

Question: How does the composition of a stacking gel differ from that of a resolving gel?

A. A stacking gel has a higher concentration of acrylamide than a resolving gel.

B. A stacking gel has a lower pH than a resolving gel.

C. There is no difference in composition between the two gels.

D. A stacking gel has the same ionic content as a resolving gel.

Answer: B

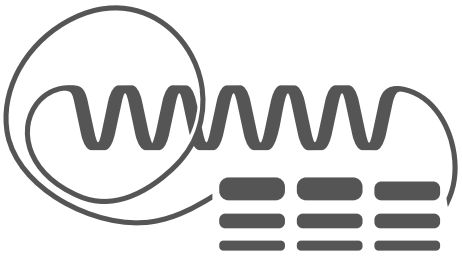
Ordering information

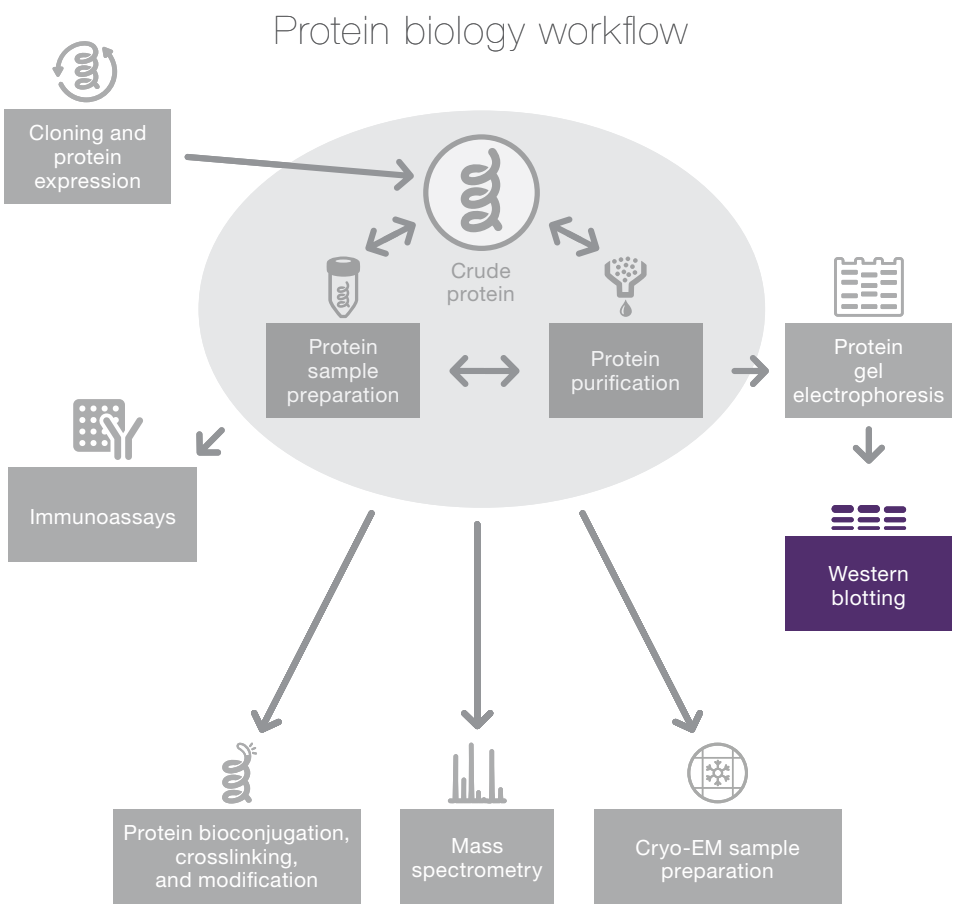
Product	Quantity	Cat. No.
SureCast welcome packs		
SureCast Gel Handcast Bundle A—Hardware and Reagents	1 kit	HC1000SR
SureCast Gel Handcast Bundle B—Hardware Only	1 kit	HC1000S
SureCast Gel Handcast Station		
SureCast Gel Handcast Station	1 casting station	HC1000
To view additional products, go to **thermofisher.com/surecast**		
Bolt Bis-Tris Plus gels		
Bolt Bis-Tris Plus mini gels*	Multiple	Multiple
Bolt welcome pack	Multiple	Multiple
Each welcome pack includes: • Mini Gel Tank • 2 boxes of Bolt Gels (2 boxes, 20 gels) • Bolt MES Running Buffer (20X), Bolt LDS Sample Buffer (4X) • Bolt Sample Reducing Agent (10X) • PageRuler Plus Prestained Ladder, 10 to 250 kDa		
* One box contains 10 gels.		
To view additional products, go to **thermofisher.com/bolt**		
NuPAGE Bis-Tris gels		
NuPAGE Bis-Tris mini gels	Multiple	Multiple
NuPAGE Bis-Tris midi gels	Multiple	Multiple
NuPAGE Tris-acetate mini gels	Multiple	Multiple
NuPAGE Tris-acetate midi gels	Multiple	Multiple
NuPAGE Bis-Tris Welcome Pack	Multiple	Multiple
To view products, go to **thermofisher.com/nupage**		
Novex Tris-glycine gels		
Novex Tris-glycine gels, WedgeWell format	Multiple	Multiple
Novex Tris-glycine plus midi gels	Multiple	Multiple
Novex Tris-glycine midi or mini welcome packs	Multiple	Multiple
Each welcome pack includes: • Novex Tris-Glycine Gels, WedgeWell format, or Novex Tris-Glycine Plus Midi Gels, 2 boxes • Novex Tris-Glycine SDS Running Buffer (10X), 500 mL • Novex Tris-Glycine SDS Sample Buffer (2X), 20 mL • NuPAGE Sample Reducing Agent (10X), 250 µL • PageRuler Plus Prestained Protein Ladder, 10–250 kDa, 2 x 250 µL • Mini Gel Tank or SureLock Tandem Midi Gel Tank		
To view additional products, go to **thermofisher.com/novexwedge**		
To view all protein gels welcome packs, visit **thermofisher.com/proteingelwelcome**		
Protein standards		
PageRuler Plus Prestained Protein Ladder	2 x 250 µL	26619
PageRuler Prestained Protein Ladder	2 x 250 µL	26616
Spectra Multicolor Broad Range Protein Ladder	2 x 250 µL	26634
Spectra Multicolor High Range Protein Ladder	2 x 250 µL	26625
HiMark Prestained Protein Standard	250 µL	LC5699
PageRuler Unstained Protein Ladder	2 x 250 µL	26614
PageRuler Unstained Low Range Protein Ladder	2 x 250 µL	26632
iBright Prestained Protein Ladder	2 x 250 µL	LC5615
MagicMark XP Western Protein Standard	250 µL	LC5602
NativeMark Unstained Protein Standard	5 x 50 µL	LC0725
To view additional products, go to **thermofisher.com/proteinladders**		

Product	Quantity	Cat. No.
Gel stains		
PageBlue Protein Staining Solution	1 L	24620
SimplyBlue SafeStain	1 L	LC6060
Imperial Protein Stain	1 L	24615
Pierce Silver Stain Kit	1 L	24612
SilverXpress Silver Staining Kit	1 kit	LC6100
SYPRO Orange Protein Gel Stain	500 µL	S6650
SYPRO Red Protein Gel Stain	500 µL	S6653
SYPRO Ruby Protein Gel Stain	1 L	S12000
Pro-Q Emerald 488 Glycoprotein Gel and Blot Stain Kit	1 kit	P21875
Pro-Q Diamond Phosphoprotein Gel Stain	1 L	P33300
To view additional products, go to **thermofisher.com/proteinstains**		
Electrophoresis chamber systems and power supplies		
Mini Gel Tank	1 unit	A25977
XCell *SureLock* Mini-Cell	1 unit	EI0001
SureLock Tandem Midi Gel Tank	1 each	STM1001
XCell4 *SureLock* Midi-Cell	1 each	WR0100
PowerEase Touch 350W Power Supply (115 VAC)	1 each	PS0350
PowerEase Touch 120W Power Supply (115 VAC)	1 each	PS0120
PowerEase Touch 600W Power Supply (115 VAC)	1 each	PS0600
To view additional products, go to **thermofisher.com/chambersystems**		
To view all electrophoresis power supplies, go to **thermofisher.com/powersupplies**		

Western blotting

Western blotting was introduced in the late 1970s and is now a routine and fundamental technique for protein analysis. Western blotting, also called protein blotting or immunoblotting, uses antibodies to identify specific protein targets after they have been separated by electrophoresis and transferred to a membrane. The specificity of the antibody–antigen interaction enables a target protein to be detected in a complex protein mixture, such as cell or tissue lysate. Western blotting can be used to generate qualitative and semi-quantitative data for a protein of interest.





Transfer

Following electrophoresis, the proteins must be transferred from the polyacrylamide gel to a membrane, typically using electroelution, or electrophoretic transfer, because of its speed and transfer efficiency. This method uses the electrophoretic mobility of proteins to transfer them from the gel to the membrane. Electrophoretic transfer of proteins involves placing the gel in direct contact with a piece of nitrocellulose or other suitable protein-binding support and sandwiching this between two electrodes submerged in a conducting solution. When an electric field is applied, the proteins move out of the gel and onto the surface of the membrane, where the proteins become tightly attached. The result is a membrane with a copy of the protein pattern that was originally in the gel. Efficiency can vary dramatically among proteins; it also depends on factors such as the composition of the gel, degree of contact of the gel with the membrane, the position of the electrodes, the transfer time, size and composition of proteins, field strength, and the presence of detergents and alcohols in the buffer.

Blocking and washing

The membranes used in western blotting have a high affinity for proteins. Therefore, after the transfer of proteins from the gel, it is important to block the remaining surface of the membrane to prevent nonspecific binding of detection antibodies during subsequent steps. The blocking buffer should improve the sensitivity of the assay by reducing background interference and improving the signal-to-noise ratio.

Use of antibodies

Western blotting is typically performed by probing the blocked membrane with a primary antibody that recognizes a specific protein or epitope. The choice of primary antibody for a western blot will depend on the antigen to be detected and the availability of antibodies for that antigen that have been verified for western blotting. In general, a primary antibody that recognizes the target protein is not directly detectable. Therefore, tagged secondary antibodies or other detection reagents are used as the means of ultimately detecting the target protein (indirect detection). A wide variety of labeled secondary detection reagents can be used; the choice of which depends upon either the species of animal in which the primary antibody was raised (the host species) or any tag on that antibody (e.g., biotin).

Following the blocking and antibody incubations steps, extensive washing is necessary to remove unbound reagents and reduce background, thereby increasing the signal-to-noise ratio. Insufficient washing will result in high background, while excessive washing may result in decreased sensitivity caused by elution of the antibody and/or antigen from the blot. As with other steps in western blotting, a variety of buffers may be used.

Detection step

While there are many different tags that can be conjugated to a secondary or primary antibody, the detection method used will limit the choice of what can be used in a western blotting assay. Alkaline phosphatase (AP) and horseradish peroxidase (HRP) are the two enzymes used most extensively as labels for protein detection. An array of chromogenic, fluorogenic, and chemiluminescent substrates are available for use with either enzyme. HRP-conjugated antibodies, which generally use chemiluminescent substrates, are considered superior to AP-conjugated antibodies with respect to the specific activities of both the enzyme and antibody, due to the smaller size of HRP and compatibility with conjugation reactions. In addition, the high activity rate, good stability, low cost, and wide availability of substrates make HRP the enzyme of choice for most applications. In well-optimized assays, chemiluminescent reactions can produce stable light output for 1–24 hours, depending on the substrate, allowing consistent and sensitive detection that may be documented with X-ray film or digital imaging equipment.

The use of fluorophore-conjugated antibodies requires fewer steps because there is no substrate development step in the assay. Recent advances in digital imaging and development of new fluorophores have increased the sensitivity and popularity of using fluorescent probes for western blotting and other immunoassays. This method has the added advantage of multiplex compatibility (using more than one fluorophore in the same experiment).

Overview of western blot transfer systems

When choosing a transfer methodology, convenience, speed, flexibility, reproducibility, and throughput are important considerations (Table 1). While the devices the laboratory currently has may be the most convenient, consider how alternative methods may improve steps such as transfer preparation and cleanup. A rapid dry transfer system, which utilizes a unique gel matrix that incorporates buffer ions instead of transfer buffer–soaked filter papers, with ready-to-use consumables, provides greater convenience, requiring very little setup and cleanup time. Regarding transfer speed, wet transfer systems are typically the slowest, followed by conventional semi-dry transfer (using Towbin buffer), with dry transfer systems being the fastest. However, with the specially designed transfer buffers for semi-dry systems, semi-dry systems can match the speeds offered by dry transfer systems. Wet tank systems come in a variety of capacities and designs and typically offer the greatest flexibility in throughput, but this attribute should be weighed against the transfer speed and potential handling inconsistencies. With respect to transfer efficiency, these modern transfer systems will transfer proteins of typical molecular weights with high efficiency. Our semi-dry systems and dry system offer preprogrammed optimized methods for particular molecular weight ranges, including high and low molecular weights. Transferring very high or very low molecular weight proteins often requires optimization regardless of the system used. However, because of the flexibility of wet transfer (e.g., transfer membranes of different pore sizes can be swapped easily and transfer buffer formulations can be modified), wet tank transfer is a good place to start when transferring proteins of very high or low molecular weights.

Table 1. Characteristics of Invitrogen™ transfer systems.

	Wet transfer		Semi-dry transfer	Dry transfer
				
	Mini Blot Module	**SureLock Tandem Midi Blot Module**	**Power Blotter Systems**	**iBlot 2 Gel Transfer Device**
Capacity	1 mini gel per blot module; 1–2 blot modules per tank	1 midi gel per blot module; 1–2 blot modules per tank	1–4 mini or 1–2 midi gels	1–2 mini or 1 midi gel
Transfer time	60 min	30 min	7–10 min	7 min
Blotting area	9 x 9 cm	9.2 x 14.4 cm	10 x 18 cm or 21 x 22.5 cm	8.5 x 13.5 cm
Transfer buffer volume	220 mL per blot module	300 mL per blot module	Pre-cut membranes and filters: 50–100 mL; pre-assembled Select transfer stacks: buffer not required	Buffer not required
Power supply	External	External	Internal	Internal
Required equipment	Mini Gel Tank	SureLock Tandem Midi Gel Tank	–	–

Learn more about the western blot transfer process in the Western Blotting Handbook at **thermofisher.com/westernhandbook**

Wet transfer systems

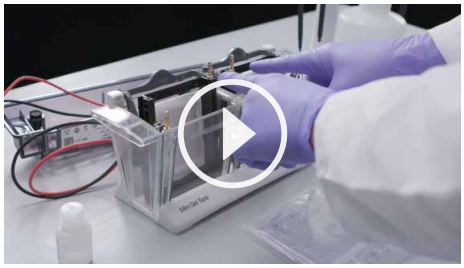
Mini Blot Module

The Invitrogen™ Mini Blot Module is a wet transfer device used exclusively with the Mini Gel Tank and is designed to make your western blot transfers simple and easy to perform. The tank accommodates one Mini Blot Module per chamber, or two blot modules total in the side-by-side layout. The universal connection and molded gasket make the blot module easy to use, while the inner core of the blot module allows for use of less methanol-based transfer buffer per wet transfer than other commercially available transfer systems. At the recommended conditions and constant voltage, proteins can typically be transferred to nitrocellulose or PVDF membranes in 30 to 60 minutes.

Highlights:

- **Universal module design**—allows modules to fit in either chamber of the tank, simplifying the transfer setup
- **Unique gasket seal**—helps prevent buffer leakage to minimize mess during setup of your western blot transfer
- **1/2-inch buffer chamber**—requires only 220 mL per blot of methanol-based transfer buffer, helping save you money on buffer and disposal costs
- **Standard 60-minute transfer protocol**—accelerates your workflow so you can get results faster
- **Robust electrodes, sturdy steel plates**—for highly efficient and reliable western blot transfers

View the how-to video:



SureLock Tandem Midi Blot Module

The Invitrogen™ SureLock™ Tandem Midi Gel Tank is uniquely designed to enable convenient, reliable gel electrophoresis and protein transfer using high-performance Invitrogen™ midi gels. With the SureLock Tandem Midi Blot Module, the tank performs efficient room temperature wet transfers for downstream western blot analysis in 30 minutes. The tank accommodates two blot modules, allowing transfer of one or two gels at a time. The unique design of the SureLock Tandem Midi Blot Module uses considerably less transfer buffer (only ~300 mL per transfer) than other midi-size wet transfer systems. This lower buffer requirement reduces the amount of hazardous methanol waste.

Highlights:

- **2-in-1 midi gel electrophoresis and transfer tank**—separate and transfer proteins in the same tank using high-performance Invitrogen midi gels
- **Two separate gel chambers**—run 1 or 2 gels or transfers using only the necessary amount of buffer for each gel, minimizing buffer cost and waste
- **Optimal performance with fast transfer protocols**—efficient, room temperature transfers in 30 minutes; eliminate the need to prechill buffers and the hassle and messiness of ice baths

View the how-to video:

Learn more at **thermofisher.com/wettransfer**

Rapid transfer systems

Power Blotter systems

The Invitrogen™ Power Blotter is our flexible solution for western blot transfer. Designed for rapid 5–10 minute semi-dry transfer of proteins from polyacrylamide gels to nitrocellulose or PVDF membranes, the Power Blotter contains an integrated power supply, LCD touchscreen, and preprogrammed, optimized transfer protocols. Harness the power of the Power Blotter in your western blotting workflow.

Highlights:

- **User-friendly**—all-in-one transfer system, with an integrated power supply and touchscreen
- **Versatile**—our most flexible transfer device, with options in throughput, consumables, and transfer speed, designed to grow around your lab's needs
- **Efficiency**—high transfer efficiency for a broad range of protein sizes (Figure 1)

Figure 1. Power Blotter Select Transfer Stacks and Power Blotter Pre-cut Membrane and Filter stacks efficiently transfer high, medium, and low molecular weight proteins. Western blot analysis of KLH, EGFR, Hsp90, PDI, and cyclophilin B was performed by loading serial dilutions of KLH-spiked HeLa lysate with KLH spike (starting at 7.5 µg HeLa lysate, 7.5 µg KLH spike per well, serially diluted 2:3) into Invitrogen™ Bolt™ 4–12% Bis-Tris Plus gels. Proteins were transferred for 7 minutes using a Power Blotter Select Transfer Stack (left) or Power Blotter Pre-cut Membrane and Filter stack (right), and then probed with target-specific primary antibodies and fluorescently conjugated secondary antibodies. Images were captured using automatic exposure on an Invitrogen™ iBright™ FL1000 Imaging System.

View the how-to video:

Learn more at **thermofisher.com/powerblotter**

iBlot 2 Gel Transfer Device

The Invitrogen™ iBlot™ 2 Gel Transfer Device is for dry electroblotting of proteins from mini-, midi-, and Invitrogen™ E-PAGE™ gels onto nitrocellulose or PVDF membranes for western blotting. The iBlot 2 system offers exceptional transfer efficiency, convenience, and speed, producing crisp and clear bands that remain sharp and straight (Figure 2). Buffer ion reservoirs incorporated into the gel matrix (transfer stacks as opposed to buffer tanks or soaked filter papers) enable rapid protein transfer to either nitrocellulose or PVDF membranes. The shortened distance between electrodes, along with high field strength and current, reduces run times to 7 minutes. With the iBlot 2 system, there is no need to prepare buffers, pretreat your gel, presoak filter papers, or clean up after blotting.

Highlights:

- **Rapid transfer**—engineered for protein transfer in just 7 minutes
- **Minimal preparation and cleanup**—transfer stacks streamline transfer setup and teardown
- **Convenient**—touchscreen interface, preprogrammed and optimized transfer protocols, and prepackaged ready-to-use stacks for transferring midi and mini blots (1 midi blot or 2 mini blots at a time)

View the how-to video:

Learn more at thermofisher.com/iblot2

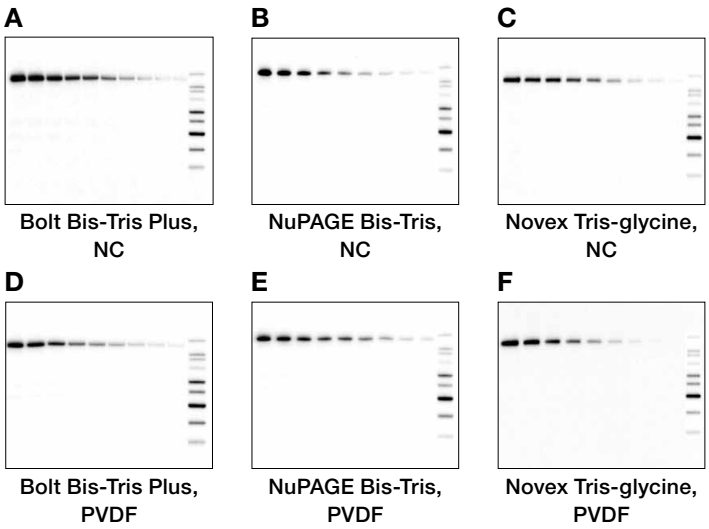


Figure 2. Membranes processed on the iBlot 2 Gel Transfer Device show consistent transfer among various protein gel compositions to both nitrocellulose (NC) and PVDF membranes. Total cell extracts from A431 cells were transferred from Invitrogen™ 4–12% Bolt™, 4–12% NuPAGE™, and 4–20% Tris-glycine precast gels to NC membranes **(A–C)** and to PVDF membranes **(D–F)**.

For more information, or to view additional products, go to thermofisher.com/proteintransfer

Western blot detection

The last step in the western blot workflow, after the separation of proteins by PAGE and their transfer from gel to membrane, is detection. In this step, primary antibodies specific to the protein of interest bind the protein on the membrane. With a variety of detection techniques to choose from (chemiluminescent, fluorescent, or chromogenic), you can select a technology to match your experimental requirements and the instruments you have available. Quick visualization or precise quantitation, single-probe detection or multiplexing: we offer a range of reagents and instruments for western blot detection and subsequent analysis.

Blocking and wash buffers

Verified western blot antibodies

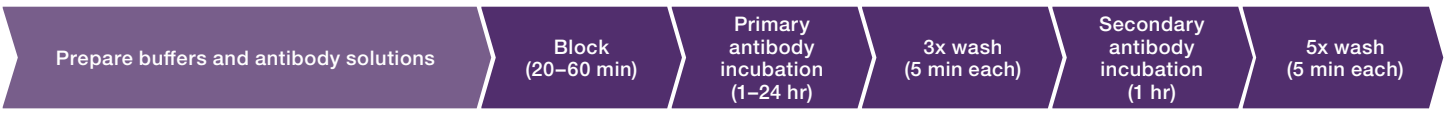
Sensitive substrates

Imaging and analysis

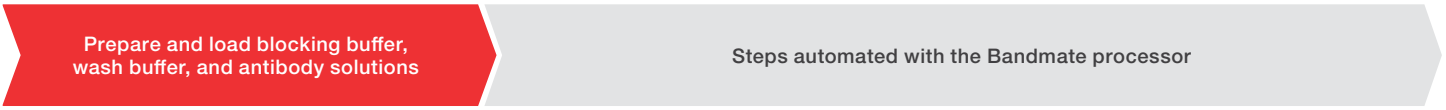
Manual vs. automated western blot processing

Traditionally, probing a western blot prior to data visualization involves a series of manual steps, many of which are individually short but collectively require significant hands-on time. We offer instruments to automate some of these tasks, tremendously decreasing hands-on time. Automated western blot processing using the Invitrogen™ Bandmate™ Automated Western Blot Processor or Invitrogen™ iBind™ or iBind™ Flex Western Device requires only 15–30 minutes of setup and no additional hands-on steps before you can move to final detection (Figure 3).

Workflow for traditional manual processing



Workflow for Bandmate processor automated processing



Workflow for iBind Western Devices processing

Figure 3. Comparison of workflows for traditional manual vs. automated western blot processing. The traditional workflow requires more than 10 hands-on steps and at least 4 hours before final detection can occur. Automated processing using the Bandmate Automated Western Blot Processor or iBind or iBind Flex Western Device requires only 15–30 minutes of setup and no additional hands-on steps before final detection.

Bandmate Automated Western Blot Processor

The Invitrogen™ Bandmate™ Automated Western Blot Processor is a programmable blot rocking system that automates the tedious hands-on blocking, washing, and antibody incubation steps of western blot processing. Minimal effort is required to set up the Bandmate device to process up to 2 midi blots or 4 mini blots using your current optimized reagents and protocols for blot processing, freeing up time for other important tasks.

- **Load-and-go setup**—prepare block, wash, and antibody solutions, load into the machine, select a program, and walk away
- **Works with traditional western blotting protocols**—no need to switch from current protocols and no specialized reagents required (Figure 4); program the timing of steps based on preference or use preprogrammed options
- **Antibody recovery**—collection tubes can recover antibody for reuse in future experiments if desired

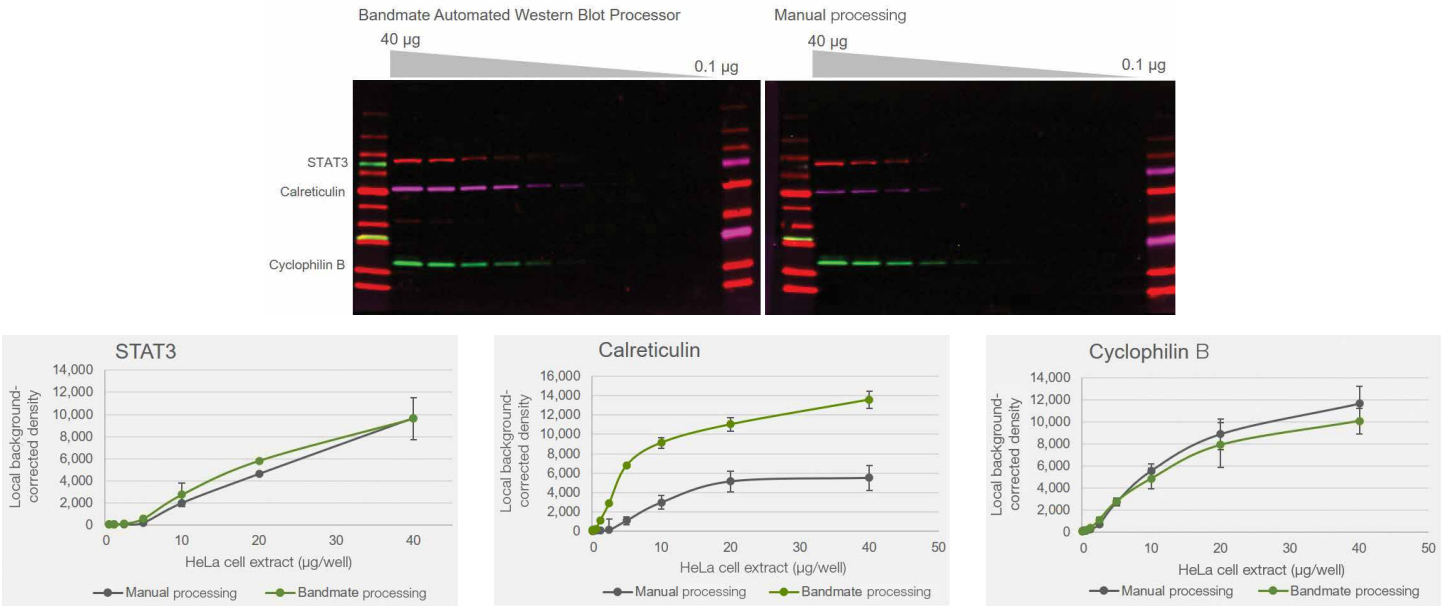


Figure 4. Comparison of mini blots processed with the Bandmate Automated Western Blot Processor vs. manual processing (probing and washing steps performed in a tray and on a shaker platform). The blots processed with the Bandmate device show comparable or higher intensity levels across multiple probed targets compared to the blots processed manually.

View the how-to video:

Learn more at **thermofisher.com/bandmate**

iBind western detection systems

Two devices, one simple technology for western blot processing

The Invitrogen™ iBind™ western blot systems are simple, unpowered devices that automate immunodetection steps. The traditional manual process involves preparing and replacing multiple antibody and wash solutions over several hours in a tray containing the blot of interest. In contrast, iBind western systems allow all solutions to be prepared and loaded in the device at the start of the procedure, with subsequent steps proceeding automatically and uninterrupted by sequential lateral flow technology (SLF), i.e., simple capillary action—no electricity or batteries are required.

The original iBind Western Device accommodates processing one mini blot at a time, and the iBind Flex Western Device accommodates processing one midi blot, two mini blots, or up to six vertically cut strips at a time for additional flexibility and throughput.

Highlights:

- **Antibody savings**—use up to 80% less primary antibody than with traditional tray-based format (Figure 5)
- **Reproducibility**—automated processing provides improved blot-to-blot consistency
- **Load and go**—process solutions using SLF technology, with no batteries, shakers, trays, or timers required

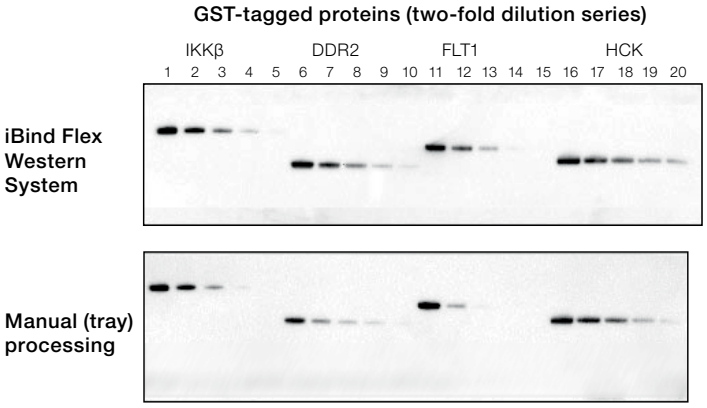
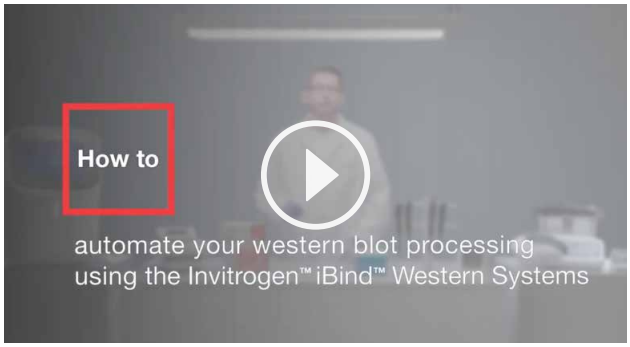


Figure 5. Comparison of western blots processed manually vs. with the iBind Flex Western Device. Samples containing GST-tagged recombinant proteins were separated on Invitrogen™ NuPAGE™ 4–12%, 20-well midi gels in MOPS SDS running buffer and then transferred to nitrocellulose membranes using the iBlot 2 Gel Transfer Device. Blots were probed with identical concentrations of the same pair of primary and secondary antibodies. The primary antibody was rabbit anti-GST diluted 1:500 (8 µL in 4 mL iBind Flex Solution for the iBind system, 40 µL in 20 mL for manual tray incubation). The secondary antibody was goat anti–rabbit IgG HRP diluted 1:600 (6.7 µL in 4 mL iBind Flex Solution for the iBind system, 33.3 µL in 20 mL for manual tray incubation). For final detection, blots were incubated for 5 minutes in Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate for visualization with an imaging system.
Lanes 1–5: IKKβ (80 ng, 40 ng, 20 ng, 10 ng, 5 ng)
Lanes 6–10: DDR2 (120 ng, 60 ng, 30 ng, 15 ng, 7.5 ng)
Lanes 11–15: FLT1 (40 ng, 20 ng, 10 ng, 5 ng, 2.5 ng)
Lanes 16–20: HCK (360 ng, 180 ng, 90 ng, 45 ng, 22.5 ng)

View the how-to video:



Learn more at **thermofisher.com/ibind**

Primary antibodies

Quality antibodies validated* and cited in many applications

The Invitrogen™ antibody portfolio has more than 200,000 antibodies cited across various applications including flow cytometry, IHC, ICC/IF, western blotting, ELISA, IP, and other applications, supporting over 50 research areas. Our primary antibody portfolio offers a broad range of choices with high-quality recombinant monoclonal, monoclonal, and polyclonal antibodies that cover greater than 91% of the proteome. With over 375,000 publications and 835,000 data images and counting, we have the antibodies you can trust.

Highlights:

- **Quality antibodies**—continued advanced specificity testing to ensure they bind to the right target (Figure 6)
- **Purchase with confidence**—use our enhanced search experience to filter and compare, so you find the data and information you need
- **More choices**—our broad portfolio allows you to easily find the antibodies you need to support your research

Commitment to antibody specificity and reproducibility

Thermo Fisher Scientific is working to redefine antibody performance standards with a comprehensive approach to how antibodies are evaluated and validated. Specificity testing is combined with extensive application validation data to provide confidence that our high-quality Invitrogen antibodies will help enable superior performance in your research.

Advanced Verification

You can easily identify antibodies that have already undergone this **additional testing** with the Advanced Verification badge. This badge can be found in antibody search results and on appropriate antibody product web pages. The **additional data** supporting the Advanced Verification status can be found in the product-specific data image galleries.

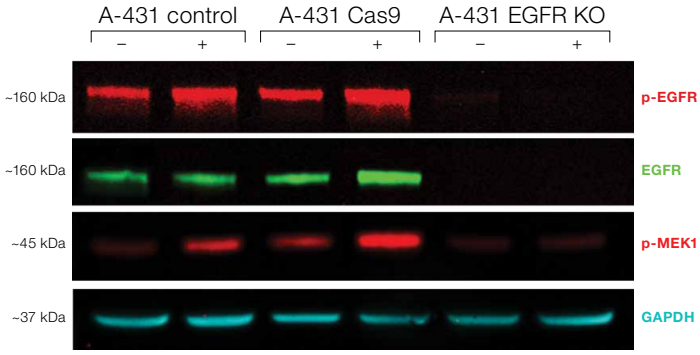


Figure 6. Western blot analysis using primary antibodies and fluorescent dye–conjugated secondary antibodies. Western blot analysis of phospho-EGFR and phospho-MEK was performed by loading 30 µg of the following whole-cell extracts: A-431 control (lane 1), A-431 treated with EGF (lane 2), A-431 Cas9 (lane 3), A-431 Cas9 treated with EGF (lane 4), A-431 EGFR KO (lane 5), A-431 EGFR KO treated with EGF (lane 6). Phospho-EGFR was detected at ~160 kDa using rabbit polyclonal antibody, EGFR was detected at ~160 kDa using mouse monoclonal antibody, phospho-MEK1 was detected at ~45 kDa using rabbit monoclonal antibody, and GAPDH was detected at ~37 kDa using goat polyclonal antibody. Target proteins were detected using Invitrogen™ Donkey Anti–Mouse IgG Alexa Fluor™ Plus 680 Conjugate, Invitrogen™ Donkey Anti–Rabbit IgG Alexa Fluor™ Plus 800 Conjugate, and Invitrogen™ Donkey Anti–Goat IgG Alexa Fluor™ Plus 555 Conjugate.

* The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic use.

Search for antibodies or find out more at **thermofisher.com/antibodies**

Secondary antibodies

Broad menu for multiple detection options

Our selection of high-quality secondary antibodies can be used for detection of the primary antibody in a broad range of applications, including western blotting, ICC/IF, flow cytometry, and ELISA. These highly cited, well-characterized secondary antibodies are linked to dyes, enzymes, and labels: including enzyme conjugates, such as HRP and AP, labels such as biotin, and classic fluorescent dyes such as FITC, RPE, and APC. We are also the premier supplier for Invitrogen™ Alexa Fluor™ and Invitrogen™ Alexa Fluor™ Plus dyes.

- **Fluorescent detection**—well suited for studies that need to address levels of protein expression or require simultaneous detection of more than one antigen
- **Chemiluminescent detection**—well suited for low-abundance proteins, and sensitivity can be further improved using our poly-HRP–conjugated secondary antibodies or enhanced chemiluminescent (ECL) substrate to deliver high femtogram–level protein detection
- **Colorimetric detection**—provides quick and easy results and does not require special equipment

We offer a wide range of antibody conjugates targeting an array of hosts and species derived from a number of sources, including a rich lineup of recombinant secondary antibodies that offer improved sensitivity. Additionally, Thermo Fisher is the only reagent provider offering Invitrogen™ Alexa Fluor™ Plus conjugates (Figure 7). Our extensive menu includes 7 colors and 11 host/target species combinations.

Highlights:

- **Avoid cross-reactivity**—use our secondary antibodies that are highly cross-adsorbed against serum proteins or immunoglobulins of other species
- **Invitrogen™ Alexa Fluor™ and Invitrogen™ Alexa Fluor™ Plus secondary antibodies**—get distinct fluorescent bands in multiplex experiments by selecting nonoverlapping spectra for a variety of conjugates using our interactive Fluorescence SpectraViewer tool
- **Sensitive detection**—use Alexa Fluor Plus secondary antibodies or the catalytic activity of our horseradish peroxidase (HRP)-conjugated antibodies in conjunction with SuperBoost™ technology for superior detection of low-abundance proteins

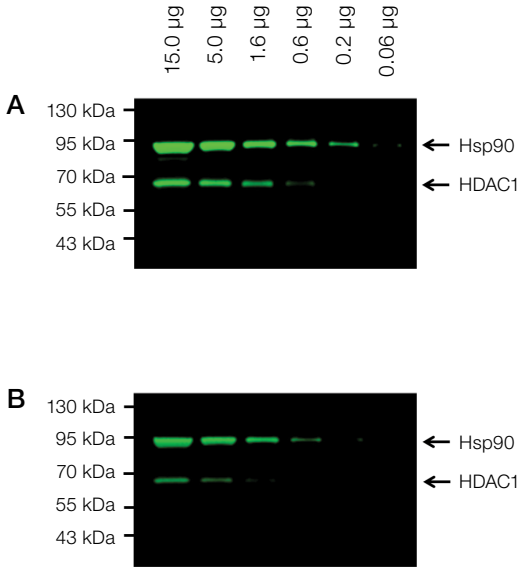


Figure 7. Comparison of detection sensitivity. **(A)** Invitrogen™ Alexa Fluor™ Plus 800 Secondary Antibody produces more bands at higher dilutions, demonstrating greater sensitivity and range of detection compared to **(B)** another supplier's fluorophore-conjugated secondary antibody.

Find out more at **thermofisher.com/alexafluorplus**

Chemiluminescent detection

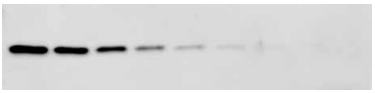
Optimized substrates to enhance sensitivity and reduce background

Thermo Scientific™ chemiluminescent HRP substrates offer excellent performance in western blotting applications with longer light emission and stronger signal intensity than other luminol-based detection systems. Choose the appropriate substrate for your needs from the Thermo Scientific™ Pierce™ ECL and Thermo Scientific™ SuperSignal™ families of chemiluminescent HRP substrates. As with other components in a western blotting system, there are many chemiluminescent substrate choices available. The appropriate substrate selection depends on the detection level (sensitivity) required, target protein abundance, and sample availability (Table 2).

Highlights:

- **Excellent sensitivity**—substrates providing picogram to attogram sensitivity
- **Strong light emission**—longer signal duration allows for multiple exposures
- **High intensity**—signal is twice as intense as other luminescence-based systems
- **Antibody savings**—our substrates are optimized to work with more dilute primary and secondary antibodies

Table 2. Recommended chemiluminescent substrates for western blot detection using HRP.

	SuperSignal West Dura	SuperSignal West Pico PLUS	SuperSignal West Atto
			
Choose when	Performing quantitative western blotting or maximum signal duration is needed	Performing everyday applications; offers improved sensitivity over base-level ECL	Target is very low-abundance, sample is limited, or antibodies are limited
Features	Longest duration and linearity	Widest dynamic range	Highest sensitivity
Detection level	Mid-femtogram	Low-picogram to femtogram	Low-femtogram to high-attogram
Signal duration	24 hours	Up to 24 hours	6 hours
Recommended antibody dilutions (1 mg/mL stock)	Primary: 1:5,000 Secondary: 1:50,000–1:250,000	Primary: 1:1,000 Secondary: 1:20,000–1:100,000	Primary: 1:5,000 Secondary: 1:100,000–1:250,000

iBright Imaging Systems

Experience an easier time capturing and analyzing data from gels and western blots with Invitrogen™ iBright™ Imaging Systems. Designed with a streamlined, intuitive interface and workflows, the iBright Imaging Systems family of instruments are easy to use for researchers of all experience levels.

There are three models in the iBright Imaging Systems family: the iBright CL750 Imaging System, the iBright CL1500 Imaging System, and the iBright FL1500 Imaging System. The iBright CL750 Imaging System offers the core essential western blot and gel imaging functions and makes the transition from the darkroom and film easy. The iBright CL1500 Imaging System expands application support and has many of the high-performance specifications of our premier iBright FL1500 model. The iBright FL1500 Imaging System features maximum application support, including fluorescent western blot imaging with up to four fluorescence channels at a time.

Highlights:

- **Push-button optimized exposure**—Smart Exposure™ acquisition technology for rapid determination of optimal exposure times helps minimize the need to repeat exposures to get the desired signal
- **Powerful 9.1 megapixel (MP) camera**—capture crystal-clear images with robust imaging potential
- **Advanced automated features**—automatic sample rotation,* automatic zoom, automatic focus, and automatic onboard data analysis provide a smooth imaging experience

- **Five-channel fluorescent blotting**—multiplex with the five fluorescence channels of the iBright FL1500 model; capture up to four proteins in a single blot for more meaningful and representative experiments
- **Compliance support**—all models offered with 21 CFR Part 11 compliance support software packages to set up and control security, audit, and e-signature settings

iBright Imaging Systems offer up to five imaging modes to support multiple applications (Table 3). Efficiently and easily capture data from protein gels, nucleic acid gels, chemiluminescent western blots, fluorescent western blots, and more (Figure 8).

* Automatic sample rotation available on CL1500 and FL1500 models.

Invitrogen™ iBright™ CL750 Imaging System

Core essential western blot and gel imaging functions to efficiently transition from the darkroom and film

Invitrogen™ iBright™ CL1500 Imaging System

Expanded application support with many of the same high-performance specifications as the premier iBright FL1500 model

Invitrogen™ iBright™ FL1500 Imaging System

Maximum application support, including fluorescent western blot imaging with up to four fluorescence channels at a time

Table 3. Imaging modes available on iBright Imaging Systems.

Imaging mode	What examples of signal can be captured?
Protein gels	Colorimetric signals from stains like Thermo Scientific™ Pierce™ Silver Stain or Invitrogen™ SimplyBlue™ SafeStain (gels) and Thermo Scientific™ Pierce™ Reversible Protein Stain or Ponceau S (membranes); fluorescent signals from stains like Invitrogen™ SYPRO™ Ruby Protein Gel Stain (gels).
Nucleic acid gels	Signals from ethidium bromide and a variety of fluorescent nucleic acid stains like Invitrogen™ SYBR™ stains.
Chemi blots	Chemiluminescence with horseradish peroxidase (HRP) and alkaline phosphatase (AP) substrates like Thermo Scientific™ SuperSignal™ and Invitrogen™ WesternBreeze™ substrates.
Fluorescent blots	Fluorescence from visible and near-infrared (NIR) fluorophores like Invitrogen™ Alexa Fluor™ and Alexa Fluor™ Plus conjugates.
Universal	Custom mode to image with multiple signals (chemiluminescent, fluorescent, colorimetric, and/or visible signals). Image display is similar to display in fluorescent blot mode and allows false colors to be assigned to any sample.

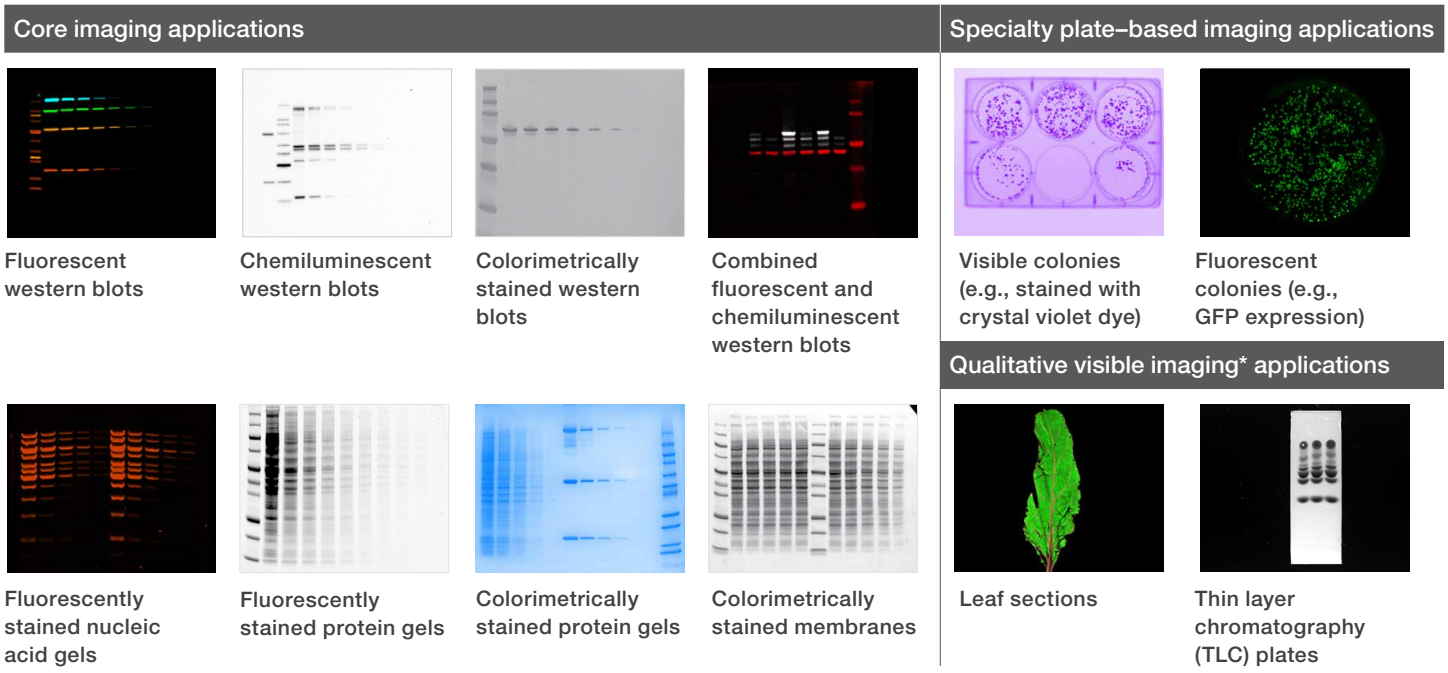


Figure 6. Examples of imaging applications. The data were captured in grayscale. Pseudocolor (false color) can be applied for visualization purposes.

* Enables qualitative visualization of samples and signal confirmation. Not recommended for quantitation.

View the overview video:

Capture your data faster and more easily than ever before. Learn more at thermofisher.com/ibright

Test your protein research knowledge

Question: Which detection method is most suitable for the detection of more than one antigen on a western blot (i.e., multiplex compatibility)?

A. Colorimetric

B. Chemiluminescent

C. Fluorescent

Answer: C

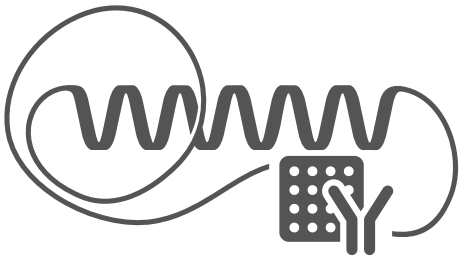
Ordering information

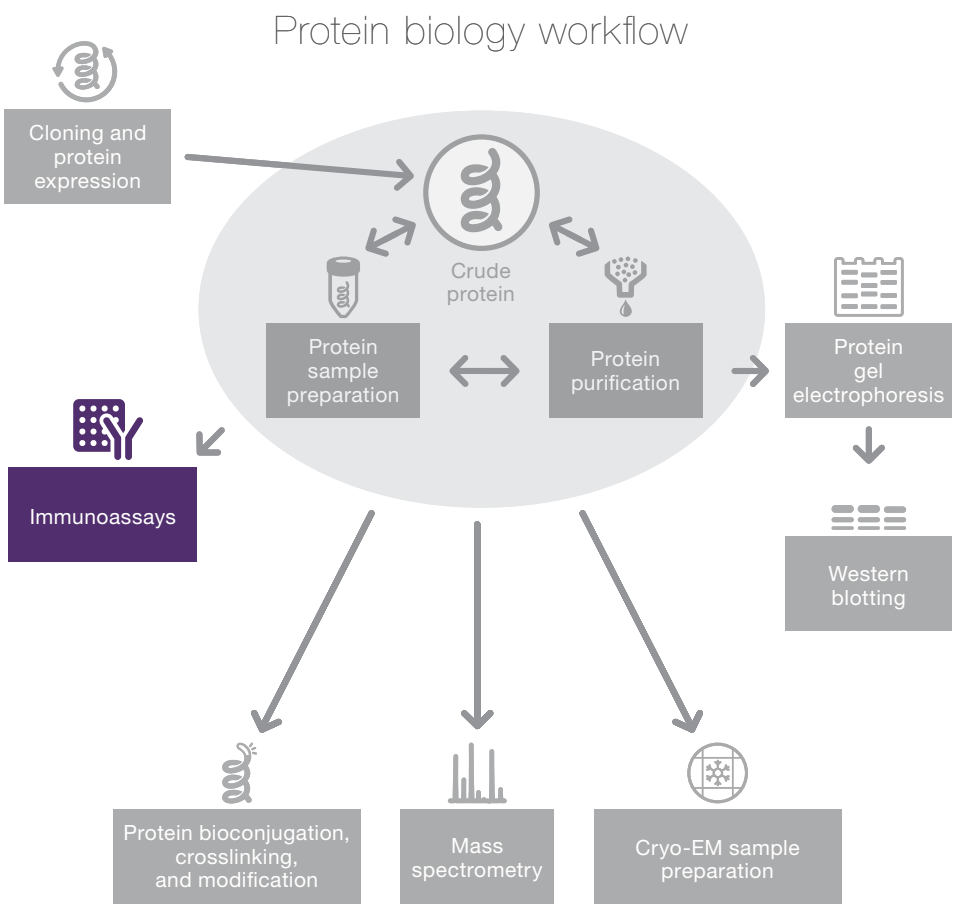
Product	Quantity	Cat. No.
Protein transfer		
Mini Gel Tank and Blot Module Set	1 kit	NW2000
Mini Blot Module	1 module	B1000
SureLock Tandem Midi Welcome Pack, nitrocellulose	1 pack	STM4015
SureLock Tandem Midi Welcome Pack, PVDF	1 pack	STM4014
SureLock Tandem Midi Blot Module	1 module	STM2001
Power Blotter XL System Welcome Pack	1 kit	PB0113
Power Blotter System Welcome Pack	1 kit	PB0112
Power Blotter XL System	1 system	PB0013
Power Blotter System	1 system	PB0012
iBlot 2 Starter Kit	1 kit	IB21001S
iBlot 2 Gel Transfer Device	1 device	IB21001
View all available products at **thermofisher.com/transfer**		
Automated western blot processing		
iBind Western Starter Kit	1 kit	SLF1000S
iBind Western Device	1 device	SLF1000
iBind Flex Western Starter Kit	1 kit	SLF2000S
iBind Flex Western Device	1 device	SLF2000
Bandmate Automated Western Blot Processor	1 system	BW1000
View all available products at **thermofisher.com/detect**		
Blocking buffers		
StartingBlock (PBS) Blocking Buffer	1 L	37538
StartingBlock (TBS) Blocking Buffer	1 L	37542
Blocker Casein in PBS	1 L	37528
Blocker Casein in TBS	1 L	37532
View all available products at **thermofisher.com/westernbuffers**		
Chemiluminescent substrates		
Pierce ECL Western Blotting Substrate	500 mL	32106
SuperSignal West Pico PLUS Chemiluminescent Substrate	500 mL	34580
SuperSignal West Dura Extended Duration Substrate	100 mL	34075
SuperSignal West Atto Ultimate Sensitivity Substrate	100 mL	A38555
View all available products at **thermofisher.com/chemisubstrates**		
Antibodies		
View all available antibodies at **thermofisher.com/antibodies**		
Imaging		
iBright CL750 Imaging System	1 instrument, 1-year warranty, and digital SmartStart Orientation	A44116
iBright CL1500 Imaging System	1 instrument, 2-year warranty, and SmartStart Orientation	A44240
iBright CL1500 Imaging System	1 instrument, 1-year warranty	A44114
iBright CL1500 Imaging System	1 instrument, 2-year warranty, SmartStart Orientation, and license for iBright SAE Software for 21 CFR Part 11	A44240CFR
iBright FL1500 Imaging System	1 instrument, 2-year warranty, and SmartStart Orientation	A44241
iBright FL1500 Imaging System	1 instrument, 1-year warranty	A44115
iBright FL1500 Imaging System	1 instrument, 2-year warranty, SmartStart Orientation, and license for iBright SAE Software for 21 CFR Part 11	A44241CFR
View all available models at **thermofisher.com/ibright**		

For more information, or to view additional products, go to **thermofisher.com/westernblot**

Immunoassays

Protein assays are an integral part of any laboratory workflow involving protein extraction, purification, labeling, or analysis. Proteins in cell lysates or purified proteins are quantified to verify yield or normalize multiple samples for side-by-side comparison. Popular protein quantitation and characterization techniques include ELISAs and Luminex® assays.





Immunoassay platforms

ELISA is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. In an ELISA, an antigen must be immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measurable product. The most crucial element of the detection strategy is a highly specific antibody–antigen interaction.

ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which will passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from unbound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

A detection enzyme or other tag can be linked directly to the primary antibody or introduced through a secondary antibody that recognizes the primary antibody. It also can be linked to a protein such as streptavidin if the primary antibody is biotin labeled. The most used enzyme labels are horseradish peroxidase (HRP) and alkaline phosphatase (AP). Other enzymes have been used as well, but they have not gained widespread acceptance because of limited substrate options. A large selection of substrates is available for performing the ELISA with an HRP or AP conjugate. The choice of substrate depends upon the required assay sensitivity and the instrumentation available for signal detection (spectrophotometer, fluorometer, or luminometer).

Though not as sensitive as fluorescent or chemiluminescent substrates, chromogenic substrates are used most frequently; they allow direct visualization and enable kinetic studies to be performed. Furthermore, chromogenic ELISA substrates are detected with standard absorbance plate readers common to many laboratories.

Invitrogen™ ProcartaPlex™ multiplex immunoassays are based on Luminex® xMAP® technology, a bead-based multiplexing technology in which beads are internally labeled with fluorescent dyes to produce a specific spectral address. Instead of attaching capture antibodies to a plate surface, capture antibodies are conjugated to the surface of beads. This technology uses flow cytometric or imaging methods for identification of the beads as well as detection of the bound antigens by the associated reporter molecules labeled with different dyes. The Luminex technology enables multiple proteins to be detected simultaneously in each well of a 96-well plate, using a very small sample volume.

Biomarker quantitation assay platforms

Highly referenced kits you can trust

To facilitate the investigation of inflammatory and other relevant biological markers, the Invitrogen™ portfolio has a broad menu of immunoassays to easily detect and quantify proteins. Options are available for single- and multi-biomarker quantitation assays, as well as the appropriate instrument platforms for each type of assay, so you can choose the platform you need to publish with confidence.

Tailored to meet your needs

A variety of assay platforms are available including plate- and bead-based solutions across many different species. ELISA kit formats include complete, ready-to-use kits, as well as preoptimized reagents to make your own. In addition, a variety of singleplex and multiplex panels for protein quantitation with Invitrogen™ ProcartaPlex™ assays are available. See Table 1 for a performance comparison of our immunoassay platforms and Table 2 for a component list.

- **Invitrogen™ coated ELISA kits**—quantitate with confidence. Highly verified ELISA kits with precoated plates provide lower inter- and intra-assay variability with ready-to-use reagents that help ensure consistent data.
  - **Invitrogen™ Instant ELISA™ kits**—the one-wash Instant ELISA kit introduces fewer handling steps and requires lower hands-on time to increase productivity.
  - **Invitrogen™ phosphospecific ELISA kits**—measure phosphospecific proteins in cell lysates.
- **Invitrogen™ antibody pair kits**—keep costs low with our affordable coat-it-yourself ELISA plate sets. Each set contains the reagents required to prepare and run the ELISA, including ELISA-optimized matched antibody pairs, standards, detection reagents, coating buffers, sample diluent, and tetramethylbenzidine (TMB) substrate solution. Plates are optional.
- **Invitrogen™ ProQuantum™ high-sensitivity (HS) assay kits**—take advantage of a platform innovation that provides researchers with an easy-to-run, high-performance assay, utilizing proximity-based amplification technology to combine the analyte specificity of high-affinity antibody–antigen binding with the signal detection and amplification capabilities of real-time PCR to achieve a highly sensitive protein quantitation assay, using very small sample volumes.
- **ProcartaPlex multiplex immunoassays**—quantitate more with less sample. ProcartaPlex multiplex immunoassays utilize Luminex xMAP technology for profiling up to 80 analytes in a single 25–50 µL sample.

Table 1. Characteristics of our immunoassay kits.

	Type of immunoassay					
	Build-it-yourself immunoassays	Uncoated ELISAs and antibody pair kits	Coated ELISA kits*	Instant ELISA kits	ProQuantum HS kits	ProcartaPlex immunoassays**
Ready-to-use reagents	No; need overnight coating process	Yes; need overnight coating process	Yes	Yes	Yes	Yes
Analytical sensitivity†	<10 pg/mL	<10 pg/mL	<10 pg/mL	<5 pg/mL	<0.1 pg/mL	<10 pg/mL
Dynamic range†	<5–250 pg/mL	<5–500 pg/mL	<5–250 pg/mL	<7.8–500 pg/mL	0.064–5,000 pg/mL	<5–2,000 pg/mL
Sample volume	10–100 µL	10–100 µL	10–100 µL	25–50 µL	2–5 µL	25–50 µL
Incubation time†	4 hr	3–4.5 hr	2.5–4 hr	4 hr	2 hr	3.5 hr
Ability to multiplex	No	No	No	No	No	Yes
Number of targets measured per well	1	1	1	1	1	1–80
Readout	HRP-TMB (colorimetric)	HRP-TMB (colorimetric)	HRP-TMB (colorimetric)	HRP-TMB (colorimetric)	FAM™ (fluorescent)	RPE (fluorescent)
Instrumentation needed	Microplate reader	Microplate reader	Microplate reader	Microplate reader	RT-PCR	Luminex® instrument
Instrument read time	2 min	2 min	2 min	2 min	36–72 min	20–60 min

* Values in this table refer to standard colorimetric kits. Ultrasensitive kits are also available.

** Immunoassays for the Luminex platform.

† Every assay has its own specifications. Please consult the protocol for your specific immunoassays or kits.

Table 2. List of components for different immunoassay kits.

	Type of immunoassay				
Immunoassay components	Antibody pair ELISA kits	Coated ELISA kits	Instant ELISA kits	ProQuantum HS kits	ProcartaPlex assays (all platforms)
96-well plate	Optional	•	•	NA	Flat bottom
Pretitrated capture antibody	•	Precoated	Precoated	•	Precoated capture bead
Pretitrated detection antibody	•	•	•	•	•
Recombinant protein standard	•	•	Additional well strips included	•	•
Stop solution	Not included	•	•	NA	NA
Substrate solution	•	•	•	NA	SA-PE
Assay buffer	•	•	Sample diluent	•	•
Wash buffer	Not included	•	•	NA	•
Coating buffer	•	NA	NA	NA	NA

Note: Our immunoassays are available in various kit sizes. Kits are calibrated to National Institute for Biological Standards and Control (NIBSC) or World Health Organization (WHO) standards (if available).
SA = streptavidin, PE = phycoerythrin, NA = not applicable.

Go to **thermofisher.com/immunoassays** to find:

- Our helpful immunoassay selection guide that allows you to search for assays based on your target protein
- Detailed information on all of our antibody pair kits, ELISA kits, and ProQuantum high-sensitivity and multiplex assay kits
- Important data demonstrating our assay specificity and sensitivity

Single-biomarker quantitation of protein targets

Precoated ELISA kits

Our ELISA kits are developed to meet specifications typically expected by customers, including standard calibration, precision, sensitivity, specificity, recovery, lot-to-lot consistency, linearity, and parallelism (Table 3).

Table 3. Rigorous assay verification of ELISA kits helps ensure consistent, reliable results.

Specification	Description	What does it mean for you?
Standard calibration	Calibrated to NIBSC, if available	Allows accurate quantitation and consistent standard of reference across multiple platforms
Precision	Average inter-assay CV <10%; average intra-assay CV <10%	Consistent results
Sensitivity	Relevant levels of protein are detected for specific assay type	Enables detection of low levels of protein
Specificity	Cross-reactivity tests are performed with similar analytes	Helps to ensure accurate measurement of the protein of interest
Recovery	Buffers are optimized to minimize matrix effects	Helps to ensure accurate quantitation of samples within a complex matrix, including serum and plasma
Lot-to-lot consistency	In-house controls are tested to measure within set ranges	Helps to ensure consistent results with new lots
Linearity of dilution	Linear results over the quantitative range of the assay	Serial dilution of samples are quantitated accurately
Parallelism	Recombinant protein standards mimic native proteins	Samples can be measured with confidence

Coated ELISA kits that include ready-to-use reagents

Quantitate with confidence

Coated ELISA kits for the quantitation of individual analytes from a variety of sample types provide lower inter- and intra-assay variability. Developed and manufactured for labs that require verified and comprehensively tested kits, coated ELISA kits offer lot-to-lot consistency and help ensure the highest possible ELISA performance (see an example standard curve in Figure 1). Ready-to-use reagents, including precoated plates, help ensure consistent data throughout your research.

Highlights:

- **Robust**—low inter- and intra-assay coefficient of variation (CV)
- **Easy to use**—includes all required assay buffers and reagents, and a straightforward protocol
- **Highly verified**—optimized for serum, plasma, and cell culture supernatants

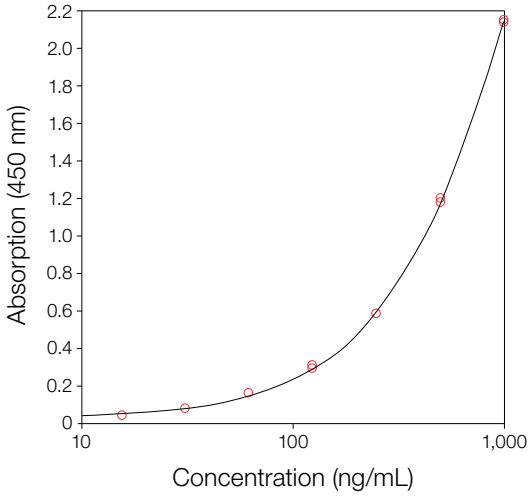


Figure 1. Standard curve for human IgG1 ELISA.

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Instant ELISA: the one-wash ELISA kit

Reduce steps and hands-on time by as much as 60%

The one-wash Instant ELISA kit requires fewer handling steps than the conventional ELISA kit (Table 4). The Instant ELISA kit contains all of the necessary components, including capture antibody and lyophilized detection antibody, streptavidin-HRP, and sample diluent. Additionally, the strip wells containing the standards for the standard curve are provided separately; they are ready to use, helping save both time and wells. Less hands-on time can help increase productivity, which leaves more time for your research.

Table 4. Steps for conventional ELISA vs. Instant ELISA kits.

17 steps for conventional ELISA		7 steps for Instant ELISA kits	
1	Washing of coated plate		
2	Reconstitution of standard protein		
3	Addition of sample diluent to standard wells		
4	Titration of standard curve	1	Rehydration of plate
5	Addition of sample diluent	2	Sample addition
6	Sample addition	3	Incubation
7	Dilution of biotin conjugate		
8	Addition of biotin conjugate		
9	Incubation		
10	Preparation of streptavidin-HRP conjugate		
11	Washing step		
12	Addition of streptavidin-HRP conjugate		
13	Incubation		
14	Washing step	4	Washing step
15	Addition of TMB substrate	5	Addition of TMB substrate
16	Addition of stop solution	6	Addition of stop solution
17	Calculation of results	7	Calculation of results

Highlights:

- **Time saving**—only requires about 15 min for setup
- **Maximum accuracy**—no need to add antibody or perform serial dilution of standards; reduced handling means less error and more consistent results
- **Better value**—generate standard curve data in parallel with additional well strips provided, to enable use of all 96 wells for your samples

Switching to Instant ELISA kits

The Instant ELISA kits utilize the same antibody pairs for many analytes across all assay formats. This enables cross-platform performance and comparable data using any of our Instant ELISA kits, ELISA, and ProcartaPlex multiplex assays. For example, if you are currently using an Invitrogen™ sCD44var Coated ELISA, you can be confident in switching to the Invitrogen™ sCD44var Instant ELISA™ assay, knowing that the data generated across the two kits will remain consistent (Figure 2).

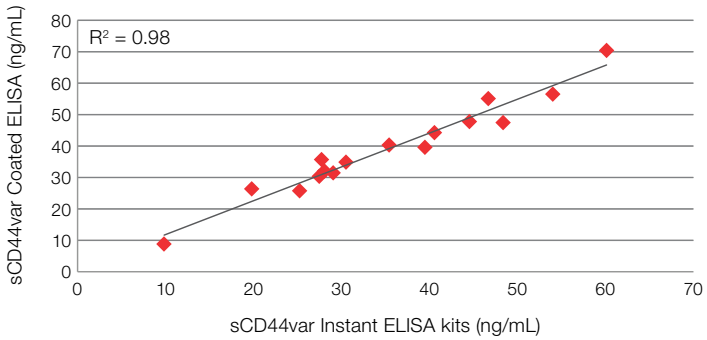


Figure 2. Performance comparison of human sCD44var Instant ELISA to coated ELISA. Human sCD44var Instant ELISA kit was tested against a human sCD44var Coated ELISA kit. A coefficient of determination (R^2) of 0.98 was observed using 16 serum samples, demonstrating comparable performance of the Instant ELISA and coated ELISA kits.

Learn more about Instant ELISA kits at thermofisher.com/instantelisa

Uncoated ELISA and antibody pair kits

Coat it yourself: optimized reagents, all in one box

Uncoated ELISA and antibody pair kits are ideal for experienced ELISA users on a budget, who have less stringent requirements for inter- and intra-assay variance. Affordable ELISA kits using coat-it-yourself plates also include the necessary reagents to prepare and run an ELISA, including ELISA-optimized matched antibody pairs, standards, detection reagents, wash buffers, and coating buffers (plates are optional).

Highlights:

- **Flexible**—purchase based on the volume of your assay through a choice of package sizes, including 2-, 10-, and 20-plate kits
- **Complete and easy to use**—includes optimized antibody pairs and recombinant standards (Figure 3), TMB substrate, and all essential reagents and buffers—unlike ELISA kits from other suppliers
- **Affordable**—priced to accommodate the most demanding budgets and to help maximize your research

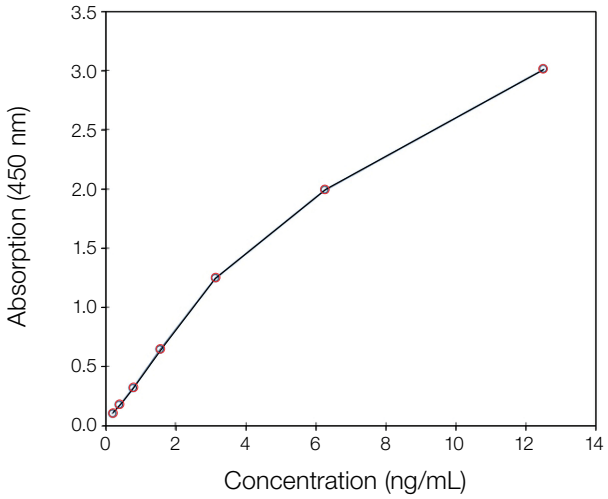


Figure 3. Representative standard curve for Invitrogen™ Mouse TGF Beta-1 Uncoated ELISA Kit with Plates.

Find out more about uncoated ELISA kits at **thermofisher.com/uncoatedelisa**

Build-it-yourself reagents

Choose from over 200,000 quality antibodies, many of them tested for ELISA, as well as buffers, reagents, and accessories to create your own immunoassay. Stand-alone products enable full flexibility to build an assay to meet your research needs.

Thermo Fisher Scientific offers a comprehensive line of accessory reagents to support your immunoassay needs. Reagents include:

- Coated and uncoated microplates
- Blocking buffers
- Diluents and wash buffers
- Substrates
- Primary and secondary antibodies
- Matched antibody pairs

Use our broad primary antibody portfolio to design your own ELISA. An important consideration in designing a sandwich ELISA is that the capture and detection antibodies must recognize two different nonoverlapping epitopes. When the antigen binds to the capture antibody, the epitope recognized by the detection antibody must not be obscured or altered. Capture and detection antibodies that do not interfere with one another and can bind simultaneously are called matched pairs and are suitable for developing a sandwich ELISA.

Use our Antigen Search tool to find antibodies to compatible binding regions. With our unique search experience, you can quickly identify the most suitable antibodies from our broad catalog.

Search for antibodies or find out more at **thermofisher.com/antibodies**

Find your optimal set of ELISA reagents using the ELISA Builder

ELISA Builder makes it easy to find the best solution for developing your own ELISA assay. Based on a short list of questions about your experimental needs, the ELISA Configurator tool recommends everything needed to perform an ELISA, from plates to stop solution.

Learn more at **thermofisher.com/elisabuilder**

See a complete listing of accessory reagents for ELISA at **thermofisher.com/elisaaccessories**

ProQuantum high-sensitivity immunoassay kits

The ProQuantum high-sensitivity immunoassay is a platform innovation that provides researchers with an easy-to-run, high-performance assay with no proprietary instrument to purchase. By utilizing proximity-based amplification technology, we have combined the analyte specificity of high-affinity antibody–antigen binding with the signal detection capabilities of real-time PCR, to achieve a highly sensitive protein quantitation assay with a large dynamic range. With its ability to detect even lower levels of protein than traditional methods with very small sample consumption, you can get the most out of your precious or limited samples.

Here's how it works

The ProQuantum immunoassays utilize a matched pair of target-specific antibodies each conjugated to a DNA oligonucleotide. During antibody–analyte binding, the two oligos are brought into close proximity, which then allows for ligation of the two strands and subsequent creation of a template strand for amplification. This proximity-based technology leverages the sensitivity and large dynamic range of Applied Biosystems™ TaqMan™ real-time PCR technology (Figure 4).

Highlights:

- **High-sensitivity**—detect low levels of proteins with greater sensitivity than with traditional ELISA methods
- **Broad dynamic range**—≥5 orders of magnitude, minimizing sample dilutions to help ensure they fall within the range (Figure 5)
- **Small sample consumption**—use 2–5 µL of sample (for example, 2 µL vs. 75 µL for triplicate wells with other methods)
- **Fast, easy workflow**—2 hr from sample to answer, no wash steps, and a single 1 hr incubation
- **No proprietary instrument to purchase**—runs on any real-time PCR instrument
- **Includes intuitive analysis software**—comprehensive data analysis and statistical groupwise comparison

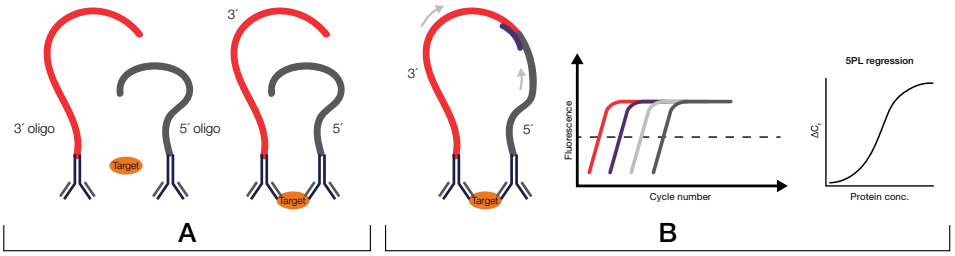


Figure 4. How ProQuantum immunoassay works. (A) Antibody conjugates binding to target (1 hr incubation). **(B)** Ligation and amplification of signal (in qPCR instrument).

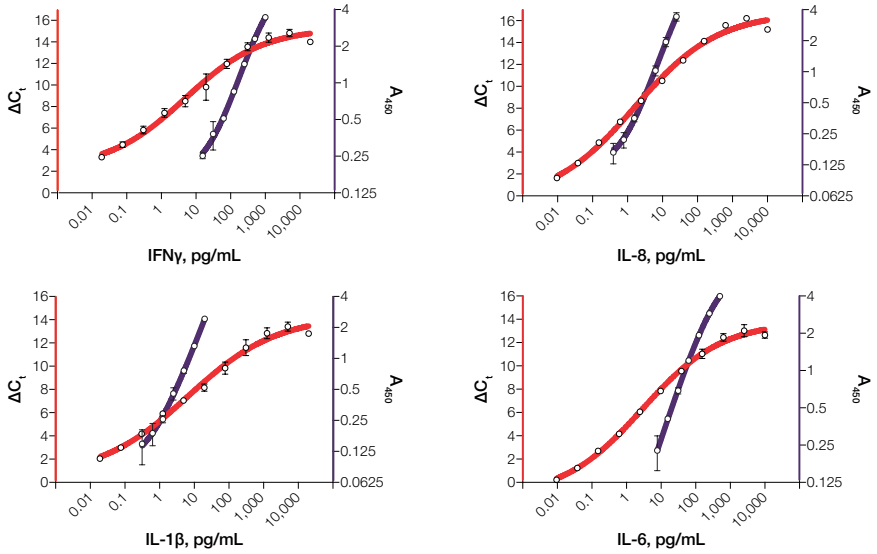


Figure 5. Superior sensitivity and broader dynamic range. Standard curves for Invitrogen™ ProQuantum™ High-Sensitivity Human IFNγ, IL-8, IL-1β, and IL-6 immunoassay kits (red) show larger dynamic range and improved separation at the low end of the curve for greater sensitivity than normal and ultrasensitive ELISAs (purple).

Find out more and select your assay at **thermofisher.com/proquantum**

Multiple-biomarker (multiplex) quantitation platforms

Protein multiplex immunoassays

Quantitate up to 80 analytes in only 25 µL of serum or plasma, or 50 µL of cell culture supernatant with ProcartaPlex multiplex assays.

Gene expression multiplex assays

Measure up to 80 RNA transcripts in a single well with Invitrogen™ QuantiGene™ Plex assays.

Luminex instruments for target quantitation

The Luminex xMAP technology combines advanced fluidics, optics, and digital signal processing with fluorescently dyed microspheres to enable the quantitation of multiple nucleic acid or protein targets from a single sample. ProcartaPlex multiplex immunoassays are designed for measurement of protein expression, and QuantiGene Plex assays are available for nucleic acid quantitation (Table 5).

High-throughput immunoassays and gene expression analysis—all from one supplier, on one instrument

As a partner of Luminex, Thermo Fisher Scientific has been providing Luminex platform users with a comprehensive offering of instruments and multiplex reagents for over 20 years. ProcartaPlex and QuantiGene Plex assays are available in 96-well and 384-well formats for high-throughput analysis.

ProcartaPlex multiplex immunoassays

ProcartaPlex multiplex immunoassays are bead-based assays for protein detection using Luminex xMAP technology. ProcartaPlex assays are based on the principles of a sandwich ELISA, using two highly specific antibodies binding to different epitopes of one protein. Invitrogen™ ProcartaPlex™ Simplex kits and panels can be easily combined to create your own panels, or we can mix and validate a panel for you.

Why use Luminex technology?

Profile more biomarkers with less starting sample: quantitate up to 80 analytes simultaneously with the INTELLIFLEX™, FLEXMAP 3D®, or Luminex® 200™ instrument, and up to 50 analytes with the MAGPIX® instrument. All of our ProcartaPlex assays are compatible with as little as 25 µL of plasma or serum, or 50 µL of cell culture supernatant.

Multiomics

Perform multiomics studies using a proven technology on a trusted platform. Hundreds of peer-reviewed publications have cited ProcartaPlex and QuantiGene Plex assays. You can have the advantage of looking at both genomics and proteomics all on one validated instrument platform. To learn more about QuantiGene assays, visit **thermofisher.com/quantigene**.

Table 5. Comparison of different multiplex assay platforms.

Assays	Analyte measured	Plex level	Targets available	Custom designs
ProcartaPlex multiplex immunoassays	Proteins	80	>500	Yes
QuantiGene Plex RNA assays	RNA	80	>17,000	Yes

Luminex xMAP technology

Quick, cost-effective, and accurate multi-analyte profiling system

The open-architecture of Luminex xMAP technology uses flow cytometry, microspheres, lasers, digital signal processing, and traditional chemistry—combining proven technologies in a unique way.

Benefits:

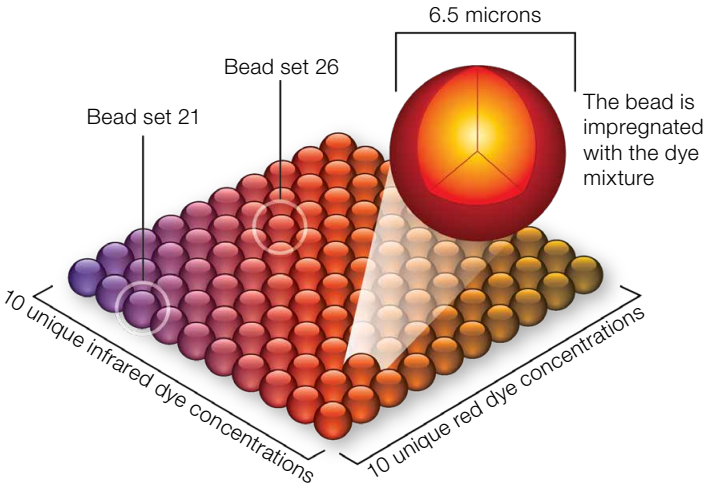
- Helps reduce costs and labor through multiplexing
- Smaller sample size requirements compared to single-result assays
- Enables fast, reproducible results from favorable kinetics of liquid bead array approach
- Broad coverage of applications, including protein expression and gene expression, to satisfy the need to understand both proteomics and genomics profiles

How Luminex xMAP technology works

Sets of magnetic microspheres are color-coded with combinations of red and infrared fluorophores. Each bead set is coated with a reagent specific to a particular biomarker, allowing the capture and detection of several specific biomarkers from a single sample. Lasers in the compact analyzer excite the internal dyes that identify each microsphere as well as any reporter dye captured, providing quantitation of individual analytes on each microsphere. The analyzer reads many beads from each set, enabling rapid and precise results for several targets within a single sample.

Luminex xMAP technology

ProcartaPlex assays have adopted the Luminex xMAP magnetic bead technology that uses Luminex® MagPlex® superparamagnetic 6.5-micron microsphere beads with a magnetic core. The beads are internally dyed with precise proportions of red and infrared fluorophores. Varying proportions of the red and infrared fluorophores result in 100 spectrally unique microspheres, which are identified by the Luminex xMAP detection systems. The conjugation of a distinct monoclonal antibody to a distinct bead allows for analysis of multiple analytes in a single well.



View the how-to video:

Watch our videos at **thermofisher.com/immunoassayvideos** to learn how to use our ProcartaPlex products.

ProcartaPlex multiplex immunoassay kits

Profile more biomarkers with less sample

ProcartaPlex multiplex immunoassays use the Luminex xMAP (multi-analyte profiling) technology that enables the simultaneous detection and quantitation of up to 80 protein targets in a single 25–50 µL sample—from plasma, serum, cell culture supernatants, and other bodily fluids. The technology employs the use of differentially dyed capture beads for each target in a multiplex ELISA-like assay. ProcartaPlex immunoassays can profile up to 80 times more analytes using significantly less sample in the same time that it takes to perform a traditional sandwich ELISA.

ProcartaPlex multiplex immunoassays are available in off-the-shelf kits or you can create your own unique panel through our mix-and-match panel offering (Table 6). It is often advantageous to start with a large panel and then reduce the number of biomarkers analyzed in a more focused panel. Whatever option works best for you, ProcartaPlex panels enable reproducible results throughout the course of your study.

- More than 90% of ProcartaPlex assays can be combined with one another
- All ProcartaPlex assays are individually tested for specificity and endogenous, native protein detection
- Multi-analyte detection with a large dynamic range
- Scalable and reproducible performance regardless of plex size
- Largest multiplex panel on the market for quantitation of up to 80 analytes

ProcartaPlex multiplex immunoassays are available in multiple formats across six species (human, mouse, rat, nonhuman primate, porcine, and canine) to meet the needs of your research.

Get information regarding characteristic assay details and individual analytes at **thermofisher.com/procartaplex**

Table 6. ProcartaPlex multiplex assay options.

Name	Description	Mixing required	Bead type	Base kit included
ProcartaPlex Preconfigured panels	Predefined, biologically relevant, and disease-defined panels using magnetic beads for the quantitative multiplex analysis of up to 80 analytes in a single sample. Optimal performance and reproducibility through extensive testing for combinability, cross-reactivity, and interference.	No	Magnetic	Yes
ProcartaPlex Mix & Match panels	Custom-blended and optimized panels deliver results tailored to the panel design of your choice. Simply provide your desired species, sample type, and instrument for use. Then select your desired analytes, and we will build and optimize a custom assay kit according to your specifications.	No	Magnetic	Yes
ProcartaPlex Simplex sets	Bead sets for the detection of individual analytes designed to be added to ProcartaPlex panels for increased customization. Alternatively, multiple ProcartaPlex simplex bead sets can be combined and run using the Invitrogen™ ProcartaPlex™ Basic Kit, which includes all non–target-specific reagents needed to perform the assay.	Yes	Magnetic	No

Have technical questions? Need help getting started?

Email **luminexfas@thermofisher.com** to get a one-on-one technical consultation.

Luminex instrumentation

The Luminex family of instruments includes options for various multiplexing capabilities, throughput, and read times (Table 7). All instruments are capable of protein and nucleic acid applications, and all are supported by commercially available assays that can multiplex up to 80 biomarkers simultaneously.

Table 7. Comparison of Luminex systems for multiplexing.




Instrument	Luminex xMAP INTELLIFLEX DR-SE System	Luminex xMAP INTELLIFLEX System	Luminex FLEXMAP 3D Instrument System	Luminex 200 Instrument System
	Most advanced Luminex platform		Higher-throughput multiplexing	Most established platform
Applications	Protein and nucleic acid analysis			
Multiplex capacity	Up to 500 targets			Up to 100 targets (80 on MagPlex microspheres)
Max panel size	80 biomarkers			
Sensitivity	0.06–1 pg/mL (immunoassays; assay and target dependent); 1,000 RNA copies per target/well (RNA assays)			
Dynamic range	≥5.5 orders of magnitude (RP1) ≥4.5 orders of magnitude (RP2)	≥5.5 orders of magnitude (RP1)	≥4.5 orders of magnitude (RP1)	≥3.5 orders of magnitude (RP1)
Sample volume (assay dependent)	6.3–80 µL			25–80 µL
Plate format	96-well 384-well			96-well
Read time	20 min (96-well) 75 min (384-well)			45 min (96-well)
Footprint	58.4 x 61 x 76 cm		110 x 62 x 63 cm	115 x 60 x 50 cm
Optics/hardware	Flow cytometry–based lasers, APDs, PMTs			
Reporter laser/optics	532 nm (green) and 405 nm (violet)	532 nm (green)	532 nm (green)	532 nm (green)
Operational validation/ compliance	Calibration, verification, and fluidics 21 CFR compliance (coming in 2022 for INTELLIFLEX instrument)			
Automation	Automation integration software available (coming in 2022 for INTELLIFLEX instrument)			
Analytics (software)	Data analysis apps for Luminex assays on the Thermo Fisher™ Connect Platform		xPONENT™ basic plus partner analytics	

Accessories to keep your Luminex instruments running strong include sheath fluid, calibrator and control microspheres, verification and calibration kits, and hand-held plate washers.

Learn more at **thermofisher.com/luminexinstruments**

Test your protein research knowledge

Question: Chromogenic ELISA assays (select all that are true):

A. Are the most commonly used ELISA detection method

B. Enable kinetic studies

C. Are as sensitive as chemiluminescent ELISAs

D. Use a standard absorbance plate reader

Answer: A, B, and D

Ordering information

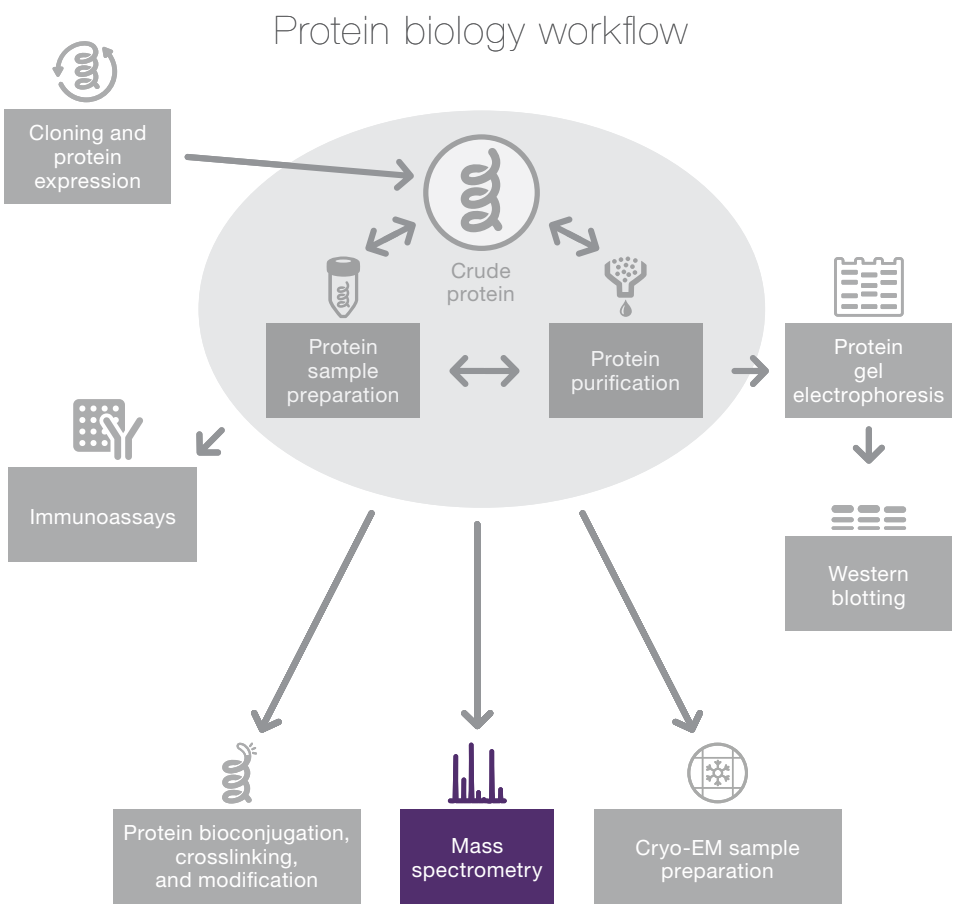
Product	Quantity	Cat. No.
ELISA kits		
Amyloid Beta 40 ELISA Kit, Human	96 assays	KHB3481
	192 assays	KHB3482
Amyloid Beta 42 ELISA Kit, Human	96 assays	KHB3441
	192 assays	KHB3442
Amyloid Beta 42 ELISA Kit, Mouse	96 assays	KMB3441
Amyloid Beta 42 Ultrasensitive ELISA Kit, Human	96 assays	KHB3544
Tau (Total) ELISA Kit, Human	96 assays	KHB0041
	192 assays	KHB0042
Tau [pS396] Phospho-ELISA Kit, Human	96 assays	KHB7031
Tau [pT181] Phospho-ELISA Kit, Human	96 assays	KHO0631
AMPK Alpha-1,2 [pT172] Phospho-ELISA Kit, Human	96 assays	KHO0651
STAT3 [pY705] Phospho-ELISA Kit, Multispecies	96 assays	KHO0481
sIL-2R/CD25 (Soluble) ELISA Kit, Human	96 assays	BMS212-2
IL-6 ELISA Kit, Human	96 assays	KHC0061
	96 assays	KAC1261
	96 assays	EH2IL6
IL-6 ELISA Kit, Mouse	96 assays	KMC0061
IL-8 ELISA Kit, Human	96 assays	KHC0081
IL-10 ELISA Kit, Human	96 assays	KAC1321
IFN Beta ELISA Kit, Human	96 assays	414101
IFN Alpha ELISA Kit, Human	96 assays	BMS216
IFN Gamma ELISA Kit, Human	96 assays	KAC1231
TGF Beta-1 ELISA Kit, Human	96 assays	BMS249-4
TNF Alpha ELISA Kit, Human	96 assays	KHC3011
	96 assays	KAC1751
TNF Alpha ELISA Kit, Mouse	96 assays	BMS607-3
	192 assays	BMS607-3TWO
SAA ELISA Kit, Human	480 assays	KHA0011C
SAA ELISA Kit, Mouse	96 assays	KMA0021
SAA ELISA Kit, Livestock	96 assays	KAA0021
Insulin ELISA Kit, Human	96 assays	KAQ1251
Leptin ELISA Kit, Human	96 assays	KAC2281
Adiponectin ELISA Kit, Human	96 assays	KHP0041
Alpha-Synuclein ELISA Kit, Human	96 assays	KHB0061
VEGF ELISA Kit, Human	96 assays	KHG0111
	192 assays	KHG0112
c-Myc (Total) ELISA Kit, Human	96 assays	KHO2041
IgG Subclass ELISA Kit, Human	192 assays	991000
Rapid ELISA Mouse mAb Isotyping Kit	60 tests	37503

To view additional products, go to **thermofisher.com/ELISA**

Product	Quantity	Cat. No.
ProcartaPlex panels		
Human		
Immune Monitoring 65-Plex Human ProcartaPlex Panel	96 tests	EPX650-10065-901
Cytokine/Chemokine/Growth Factor Convenience 45-Plex Human Panel 1	96 tests	EPXR450-12171-901
Cytokine & Chemokine Convenience 34-Plex Human Panel 1A	96 tests	EPXR340-12167-901
Cytokine Storm 21-Plex Human Panel	96 tests	EPX210-15850-901
Inflammation 20-Plex Human Panel	96 tests	EPX200-12185-901
Immuno-Oncology Checkpoint 14-Plex Human Panel 1	96 tests	EPX14A-15803-901
Coronavirus Ig Total Human 11-Plex Panel	96 tests	EPX110-16000-901
SARS-CoV-2 Variants Neutralizing Antibody 6-Plex Panel	96 tests	EPX060-16018-901
Mouse		
Immune Monitoring 48-Plex Mouse Panel	96 tests	EPX480-20834-901
Cytokine & Chemokine Convenience 36-Plex Mouse Panel 1A	96 tests	EPXR360-26092-901
Immuno-Oncology Checkpoint 7-Plex Mouse Panel 2	96 tests	EPX070-20835-901
Rat		
Antibody Isotyping 6-Plex Rat Panel	96 tests	EPX060-30123-901
Kidney Toxicity 5-Plex Rat Panel 1	96 tests	EPX050-30124-901
Canine		
Cytokine/Chemokine/Growth Factor 11-Plex Canine Panel 1	96 tests	EPX11A-50511-901
Porcine		
Cytokine & Chemokine 9-Plex Porcine Panel 1	96 tests	EPX090-60829-901
Nonhuman primate (NHP)		
Cytokine/Chemokine/Growth Factor 37-Plex NHP Panel	96 tests	EPX370-40045-901
Cytokine & Chemokine 30-Plex NHP Panel	96 tests	EPX300-40044-901
To view additional products, go to **thermofisher.com/luminex**		
ProQuantum panels		
IL-6 Human ProQuantum Immunoassay Kit	96 tests	A35573
TNF Alpha Human ProQuantum Immunoassay Kit	96 tests	A35601
IL-8 Human ProQuantum Immunoassay Kit	96 tests	A35575
IFN Alpha Human ProQuantum Immunoassay Kit	96 tests	A42897
TNF Alpha Mouse ProQuantum Immunoassay Kit	96 tests	A43658
Mouse IL-17A ProQuantum Immunoassay Kit	96 tests	A46737
To view additional products, go to **thermofisher.com/proquantum**		

Mass spectrometry

Mass spectrometry (mass spec or MS) has become a method of choice for protein analysis. The accuracy, sensitivity, and flexibility of MS instruments have enabled new applications in biological research, biopharmaceutical characterization, and diagnostic detection. MS can identify and quantify known and unknown compounds by revealing their structural and chemical properties. With its many forms of ionization and measurement, MS enables the analysis of samples ranging in mass from 50 to 300,000 Da, in attomole through nanomole quantities. Proper sample preparation and chromatography, as well as the right instrumentation and software, are critical components of successful MS-based proteomic analysis.



Protein quantitation using mass spectrometry

Differences in protein expression can be studied both globally (discovery proteomics) or within a specific subset of proteins (targeted proteomics). Most quantitative proteomic analyses utilize the isotopic labeling of proteins or peptides in the experimental groups, which can then be differentiated by mass spectrometry. Relative quantitation methods are used to compare protein or peptide abundance between samples, while spiking unlabeled samples with known concentrations of isotopically labeled synthetic peptides can help to enable absolute quantitation of target peptides via selected reaction monitoring (SRM).

Discovery quantitation strategies for relative protein quantitation include stable isotope labeling using amino acids in cell culture (SILAC) and labeling using tandem mass tag (TMT) reagents. SILAC-based quantitation involves metabolically labeling protein samples in cultured mammalian cells with a heavy isotope–labeled form of an amino acid. Inclusion of the labeled amino acid in cell or tissue culture media results in replacement of the natural light amino acid with the heavy form in newly synthesized proteins, enabling multiplexing. Isobaric chemical tags are a more universal alternative to SILAC because they can be used with a wide variety of samples including cells, tissues, and biological fluids. Isobaric chemical tags facilitate the simultaneous analysis of up to eighteen samples and consist of an MS/MS reporter group, a spacer arm, and a reactive group. Amine-reactive groups covalently bind to peptide N termini or to lysine residues. Each tag fragments during MS/MS, producing unique reporter ions, enabling multiplexed quantitation through comparing the intensities of the reporter ions.

Absolute quantitation is performed in targeted proteomic experiments and increases the sensitivity of detection for a limited number of target analytes. These approaches require spiking a sample with known amounts of synthetic peptides containing heavy stable isotopes, which act as internal quantitative standards for absolute quantitation of the corresponding natural peptides in the sample.

Sample preparation

Sample preparation is one of the most variable and time-consuming steps in the analysis of proteins by MS, and the quality and reproducibility of sample extraction and preparation significantly impact the results. Robust, integrated workflows enable consistent results between labs and help eliminate wasted time spent troubleshooting experimental methods and results.

Protein extraction, depletion, and enrichment strategies have been developed to remove high-abundance proteins or isolate target proteins in the sample, reduce sample complexity, and help improve the detection of low-abundance proteins. Digestion is required because proteins are often too big and complex for analysis and therefore are digested into peptides for MS analysis for better detection and identification of proteins. Trypsin is the protease of choice for protein digestion. However, digestion with alternative proteases such as Glu-C, Lys-N, Lys-C, Asp-N, or chymotrypsin can improve individual protein sequence coverage or generate unique peptide sequences for different MS applications.

Instrument calibration

Calibration solutions and standards are critical for optimal performance in mass spectrometry; mass spectrometers must be carefully monitored to ensure accuracy of results. Ideally, calibration reagents should be ready-to-use liquid formulations composed of highly purified, ionizable molecules or polymers specifically designed for positive or negative calibration of instruments. Standards should be designed for specific applications, including sensitivity assessment, determination of digestion efficiency, chromatography assessment, or as controls for sample analysis.

Protein quantitation

We offer reagents and kits for both discovery and targeted proteomics applications (Table 1). Discovery proteomics utilizes techniques such as SILAC and isobaric mass tagging that allow the user to look at global protein expression levels. In contrast, targeted proteomics utilizes selected or multiple reaction monitoring (SRM/MRM), which are methods of absolute quantitation (also termed AQUA), in which a complex sample is spiked with a stable isotope-labeled peptide that acts as internal standard for a specific subset of proteins.

Table 1. Choose the right protein quantitation reagent for mass spectrometry.

	SILAC	TMT	TMTpro	Peptides for SRM	SureQuant targeted assays
Application type	Discovery quantitation			Targeted quantitation	
Labeling method	Metabolic	Amine-, cysteine-, and carbonyl-reactive mass tags	Amine-reactive mass tags	Spike-in standard	Spike-in standard
Sample type	Cultured mammalian cells	Cultured mammalian cells; tissue; biofluids	Cultured mammalian cells; tissue; biofluids	Cultured mammalian cells; tissue; biofluids	Cultured mammalian cells; tissue; biofluids
Digestion protocol	In-gel	In solution	In solution	In solution	In solution
Quantitation mode	MS^1	MS^2 (6-plex) MS^3 (10-plex) MS^3 (11-plex)	MS^3 (16-plex) MS^3 (18-plex)	MS^1	MS^1
Multiplex options	2-plex, 3-plex, 4-plex	2-plex, 6-plex, 10-plex, 11-plex	16-plex, 18-plex	Samples not combined	Samples not combined

Tandem Mass Tag (TMT and TMTpro) systems

Simultaneously identify and quantify protein expression from multiple samples in a single analysis

The Thermo Scientific™ TMT™ and TMTpro™ Label Reagent Sets enable simultaneous quantitation of up to 11 (TMT reagents) or 18 (TMTpro reagents) samples, providing the highest throughput available for protein identification and quantitative analysis by tandem mass spectrometry (MS).

Highlights:

- **Multiplex**—concurrent MS analysis of up to 11–18 samples derived from cells, tissues, or biological fluids (Figures 1–3)
- **Robust**—increased multiplex capability results in fewer missing quantitative values among samples and higher confidence among replicates
- **Efficient**—amine-reactive, NHS ester–activated reagents ensure efficient labeling of all peptides regardless of protein sequence or proteolytic enzyme specificity
- **Convenient**—provided in a ready-to-use, single-use format to ensure optimal stability and performance or in bulk for custom formatting

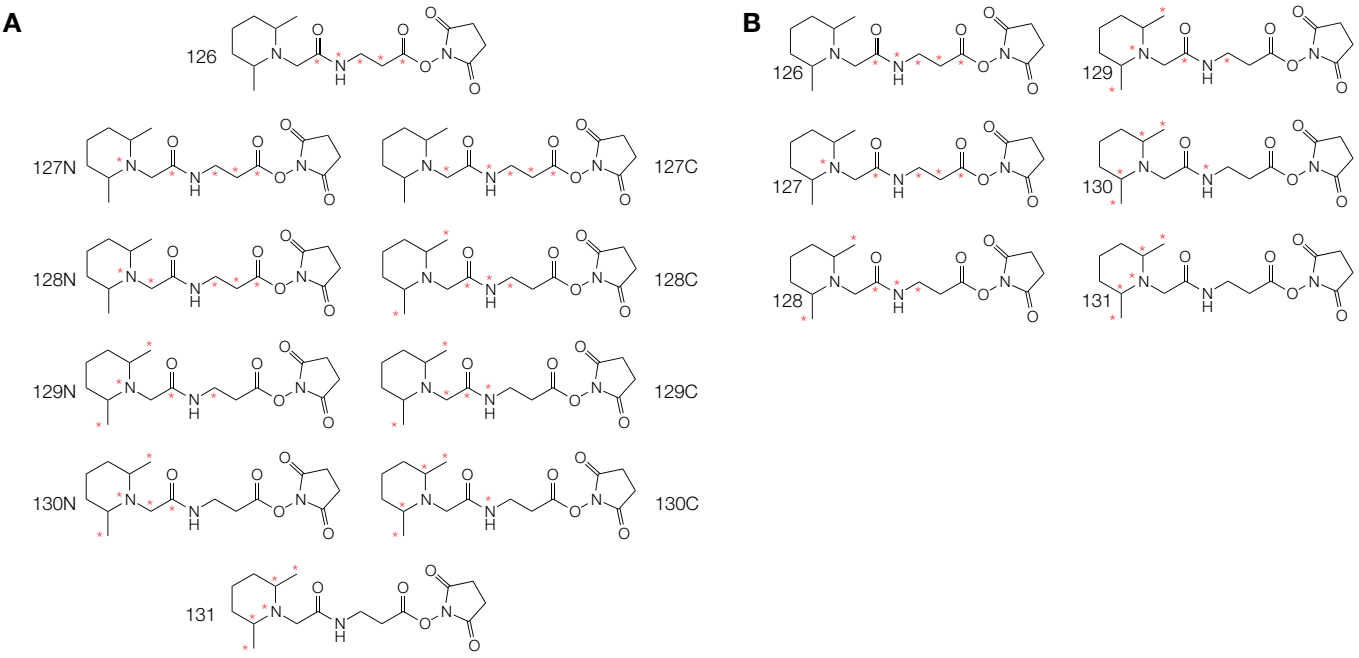


Figure 1. Chemical structures of TMT reagents. (A) Structures of TMT10plex reagents with ^{13}C and ^{15}N heavy-isotope positions (red asterisks). **(B)** Structures of TMTsixplex reagents with ^{13}C and ^{15}N heavy-isotope positions (red asterisks).

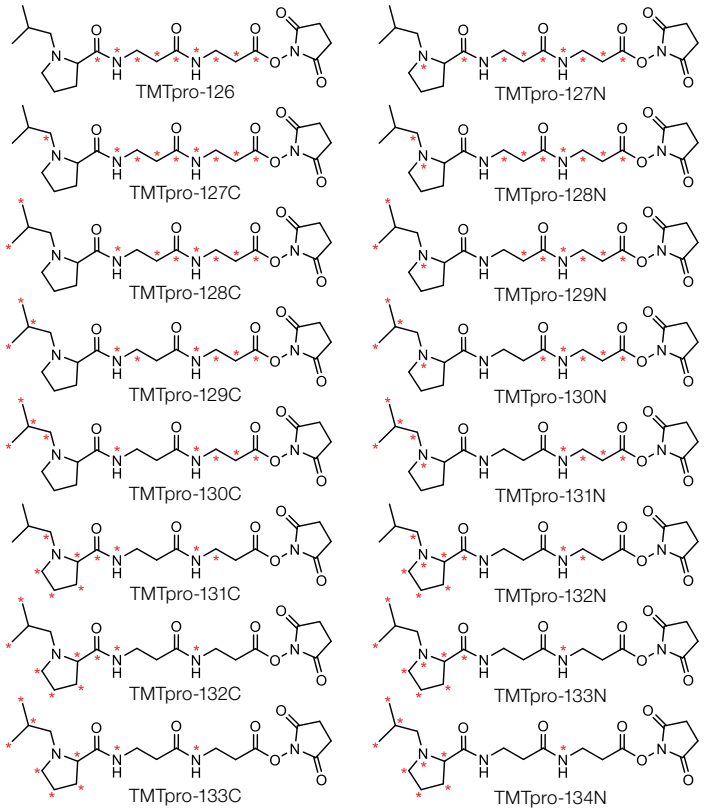


Figure 2. Structures of TMTpro 18plex reagents with ^{13}C and ^{15}N heavy-isotope positions (red asterisks).

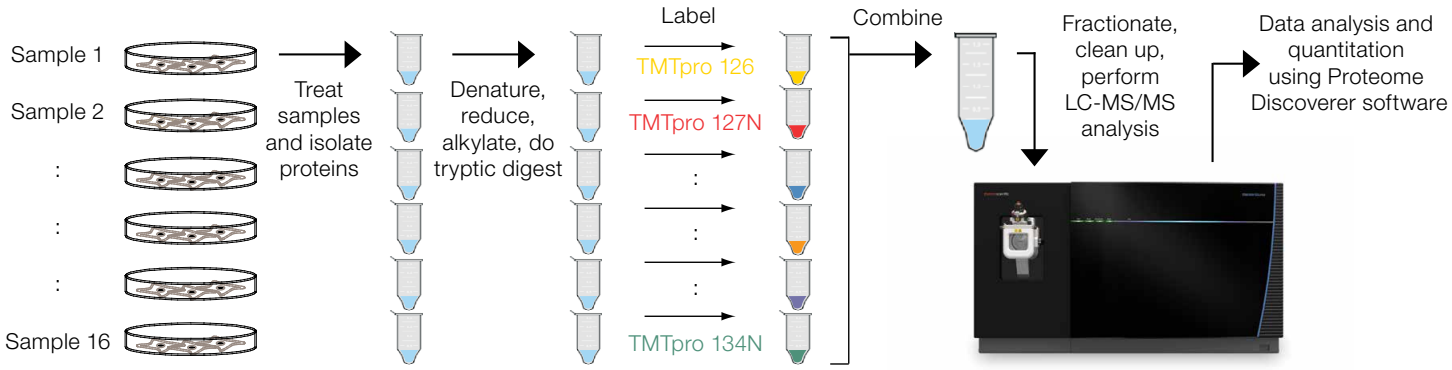


Figure 3. Procedure summary for MS experiments using TMTpro isobaric mass tagging reagents. Protein extracts isolated from cells or tissues are reduced, alkylated, and then digested using the Thermo Scientific™ EasyPep™ Mini MS Sample Prep Kit or an equivalent method. Samples are then labeled with the TMTpro reagents before sample mixing, fractionation, and cleanup. Labeled samples are analyzed on a high-resolution Thermo Scientific™ Orbitrap™ LC-MS/MS mass spectrometer before data analysis to identify peptides and quantify relative abundance of reporter ions.

Learn more at **thermofisher.com/tmtpro**

SureQuant assays and standards

Validated, modular reagents for multiplexed targeted protein quantitation

The Thermo Scientific™ SureQuant™ assays and standards have been designed for multiplexed targeted quantitation for key cellular signaling proteins. The assays provide flexibility to choose the use of the immunoprecipitation sample prep kits and peptide quantitation modules together or independently to best suit the end application. The MS sample preparation has been streamlined to be completed in ~4 hours. Together, SureQuant assays and standards provide a complete solution for multiplex IP enrichment and analysis of target proteins (Figures 4 and 5).

Highlights:

- **Complete**—includes reagents for successful sample preparation and quantitative analysis of each target peptide
- **Verified**—kits and reagents rigorously tested for specificity and successful quantitation of each target peptide
- **Multiplex**—Thermo Scientific™ HeavyPeptide™ AQUA panels for simultaneous quantitation of target proteins and phosphorylation status from key cell signaling pathways
- **Flexible**—modular format allows for immunoenrichment only, or in combination peptide quantitation panels

Learn more at **thermofisher.com/ms-targeted-assays**

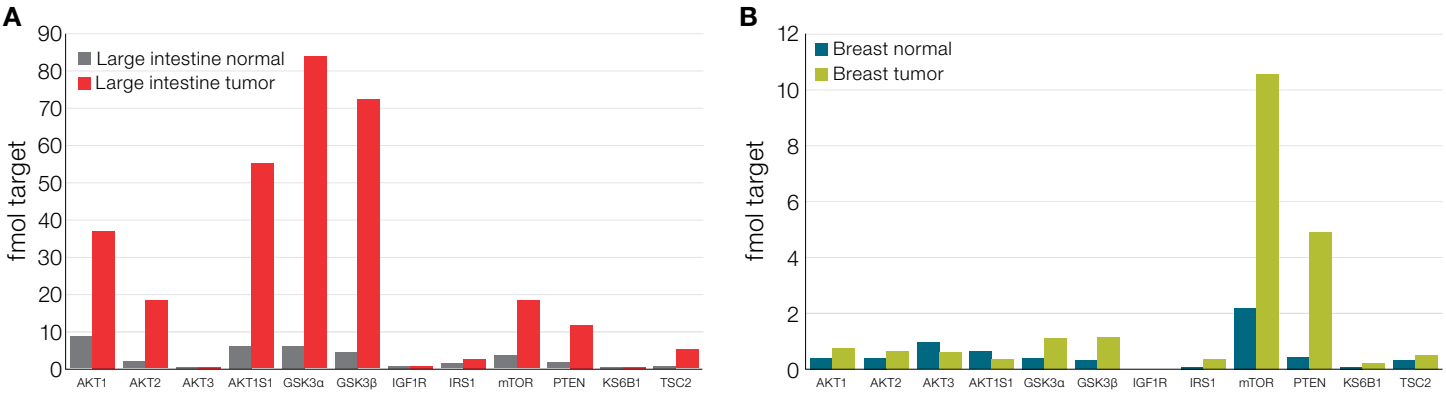


Figure 4. Multiplex IP analysis using streptavidin magnetic beads with human tissue. AKT/mTOR pathway proteins were enriched through multiplex IP using biotinylated antibodies from Thermo Scientific™ SureQuant™ AKT pathway kits. Parallel reaction monitoring (PRM) analysis for **(A)** total AKT/mTOR pathway targets and **(B)** phosphorylated AKT/mTOR pathway targets was performed using the Thermo Scientific™ UltiMate™ 3000 RSLCnano System and Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Data were subsequently analyzed in Skyline software using calibration curves to determine the absolute protein concentration (fmol).

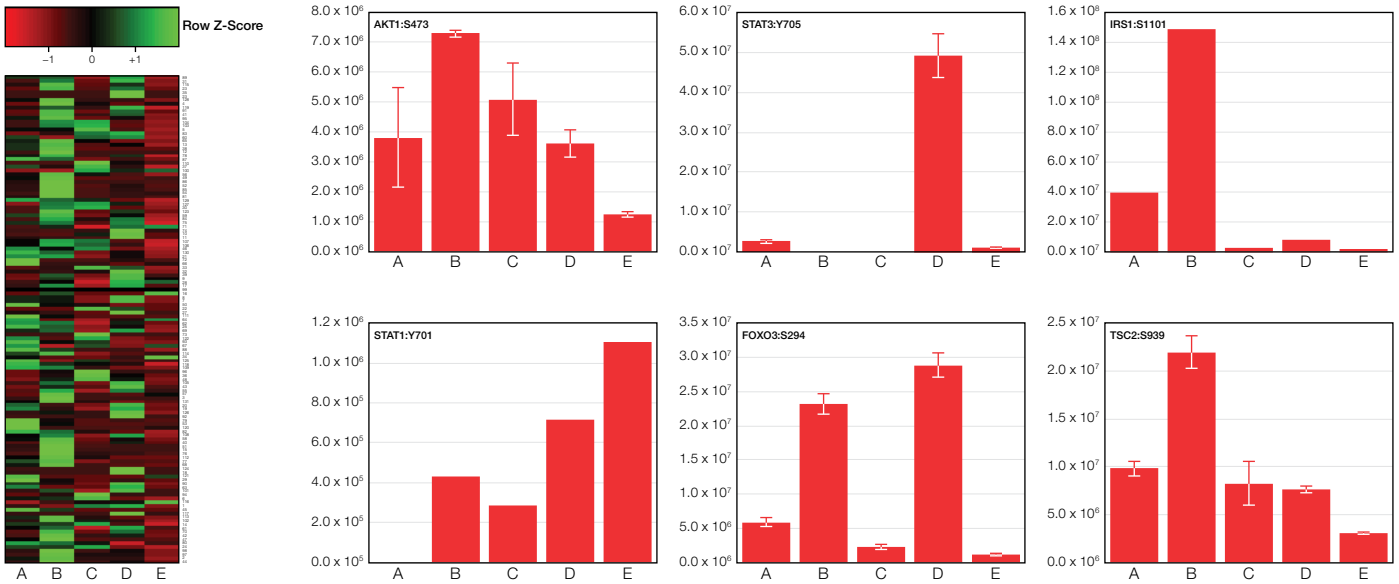


Figure 5. Use of the Thermo Scientific™ SureQuant™ Multipathway Phosphopeptide Standard with phosphopeptide enrichment enables relative quantitation of greater than 100 phosphoproteins. Heat map of relative levels of phosphopeptides **(left)**. Changes in specific phosphopeptides **(right)**. One microgram of treated cell lines was incubated with Fe-NTA magnetic beads. Prior to enrichment, 1 pmol of the SureQuant Multipathway Phosphopeptide Standard was spiked into the lysate. PRM analysis was performed using the UltiMate 3000 RSLCnano System and Q Exactive HF Hybrid Quadrupole-Orbitrap Mass Spectrometer and subsequently analyzed in Skyline software using the average total area. A: HCT116/IGF; B: MCF7/IGF; C: LNCaP/IGF; D: A431/EGF; E: HepG2/Ins.

HeavyPeptide and LightPeptide AQUA custom standards

Improve quantitative precision for absolute quantitation

The Thermo Scientific™ HeavyPeptide™ and LightPeptide™ AQUA custom synthesis services provide isotopically labeled or unlabeled AQUA (Absolute QUAntitation) peptides with extensive QC for relative and absolute quantitation of proteins. HeavyPeptide and LightPeptide standards are synthesized using the latest Fmoc solid-phase peptide synthesis technology, purified by HPLC, and analyzed by mass spectrometry. Amino acid analysis (AAA) provides the most exact peptide concentration to guarantee the highest level of quantitative precision. The different grades of AQUA peptides are defined by the replicates of AAA, with Basic (1X), QuantPro (2X), and Ultimate (3X).

Highlights:

- **Precise**—peptide concentration guaranteed from AAA
- **Sensitive**—enables absolute quantitation of low-abundance proteins (fmol)
- **Specific**—100% peptide sequence specificity
- **Flexible**—variety of purity, modification, and formatting options (Table 2)

Table 2. Specifications of HeavyPeptide AQUA-grade standards.

	AQUA Ultimate Service	AQUA QuantPro Service	AQUA Basic Service
Research stage	Validation, ideal for absolute quantitation	Confirmation or validation, ideal for biomarker verification	Discovery, confirmation or validation, relative quantitation
Amount/number of aliquots	10 nmol/10 aliquots 40 nmol/40 aliquots 96 nmol/96 aliquots	10 nmol/10 aliquots 40 nmol/40 aliquots 96 nmol/96 aliquots	15 to 30 nmol (0.05 to 0.1 mg)/1 aliquot
Formulation	5 pmol/µL in 5% (v/v) acetonitrile/H_2O	5 pmol/µL in 5% (v/v) acetonitrile/H_2O	Lyophilized
Shipment	In solution on wet ice	In solution on wet ice	Dry at room temperature
Concentration precision	±5–10%	±10–25%	NA
Peptide length*	Up to 15 amino acids		
Purity	>97%		
Isotopic enrichment	>99%		
Standard (light) peptides available	Yes		
Delivery format	Glass vial		
Production time	5–7 weeks standard AQUA		
Quality control	MALDI-TOF MS, HPLC, quantitative AAA		
Modifications	Single or double phosphorylation (pY, pT, or pS) Cysteine carbamidomethylation (CAM) Methionine sulfoxide [Met(O)] Other modifications available on request		
Optional services	Additional light amino acids to extend peptide length Additional heavy amino acids within the peptide sequence Multiple solvents, concentrations, and aliquot sizes available		

* Please inquire about longer peptide lengths.

Learn more at **thermofisher.com/heavypeptide**

Protein sample preparation

Validated reagents for robust and reproducible sample processing

Thermo Scientific™ reagents and kits are optimized for mass spectrometry sample preparation for every step of the process, from depletion strategies to reduce complexity, to protein extraction, enrichment, and cleanup for the isolation of low-abundance proteins. Post-digestion, peptide enrichment, fractionation, and cleanup steps are often critical for proper analysis. The Thermo Scientific™ Pierce™ Quantitative Colorimetric and Fluorometric Peptide Assays enable sample normalization following digestion.

Highlights:

- **Comprehensive**—reagents and kits for every step of the sample preparation workflow, both before and after protein digestion
- **Optimized**—reagents and kits are designed to maximize yield without sacrificing quality
- **Versatile**—products available as complete kits or as stand-alone reagents, featuring the Thermo Scientific™ EasyPep™ and High-Select™ lines of sample prep kits
- **Compatible**—kits are designed to be compatible with mass spectrometry analysis
- **Validated**—products have been fully tested, and processed samples have been analyzed using Thermo Scientific™ mass spectrometers

High-Select Abundant Protein Depletion Reagents and Phosphopeptide Enrichment Kits

The High-Select product line offers kits and resins for better detection of low-abundance proteins through sample depletion and enrichment. Thermo Scientific™ High-Select™ HSA/Immunoglobulin and Top14 Abundant Protein Depletion Resins deplete the abundant protein and antibody components of human plasma samples, helping to prepare the samples for mass spectrometry or 1D or 2D electrophoresis (Table 3). Thermo Scientific™ High-Select™ Phosphopeptide Enrichment Kits and Resins available in agarose and magnetic bead formats enable efficient and selective enrichment of phosphorylated peptides for mass spectrometry analysis.

Highlights:

- **Optimized**—protocols and reagents designed to maximize results in a fraction of the processing time
- **High quality**—greater than 95% depletion of the top serum/plasma proteins and phosphopeptide recovery with >90% selectivity (Figures 6–8)
- **Flexible**—kits and resins provided in several formats to address sample input and throughput
- **Scalable**—compatible with automation

Table 3. Proteins removed by High-Select abundant protein depletion spin columns.

HSA/immunoglobulin	Top14 columns	
Albumin	Albumin	α-Acid glycoprotein
IgG	IgG	Fibrinogen
IgA	IgA	Haptoglobin
IgM	IgM	α1-Antitrypsin
IgD	IgD	α2-Macroglobulin
IgE	IgE	Transferrin
IgG (light chains)	IgG (light chains)	Apolipoprotein A-1

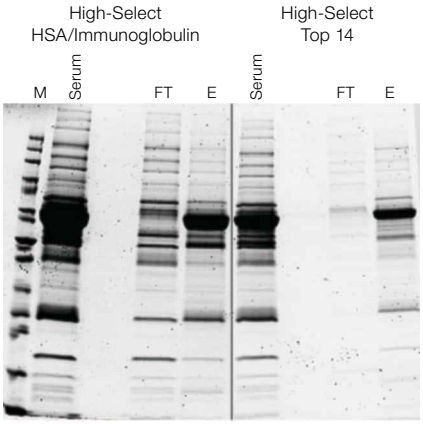


Figure 6. High-Select protein depletion spin columns remove proteins to enable visualization of low-abundance proteins. Human serum (10–20 µL) was loaded onto each resin and processed according to protocols. Total protein in the depleted fractions was estimated using the Thermo Scientific™ Pierce™ BCA Protein Assay Kit. The total amount of albumin in the depleted fractions was determined using the AssayMax™ Human Albumin ELISA Kit. FT = flowthrough (i.e., depleted sample); E = eluate (i.e., proteins that were bound by the resin, plus stripped affinity antibodies of the column). With the top 14 proteins removed, low-abundance proteins are now visible in each depleted sample lane (FT).

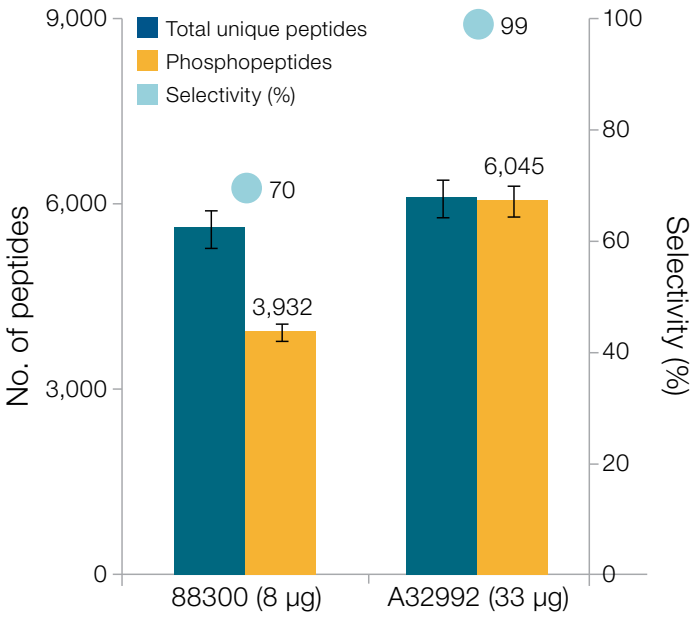


Figure 7. The High-Select Phosphopeptide Enrichment Kit (Cat. No. A32992) improves selectivity and yield over the Pierce Fe-NTA Phosphopeptide Enrichment Kit (Cat. No. 88300). The average selectivity for phosphopeptides is 99% with a 3- to 4-fold improvement in peptide yield. Protein digests (1 mg) from nocadazole-arrested HeLa cells were used for the enrichment according to instructions. Eluted peptides were analyzed using a Thermo Scientific™ EASY-Spray™ C18 LC Column (3 µm, 150 mm) on a Thermo Scientific™ Orbitrap™ Fusion Tribrid Mass Spectrometer.

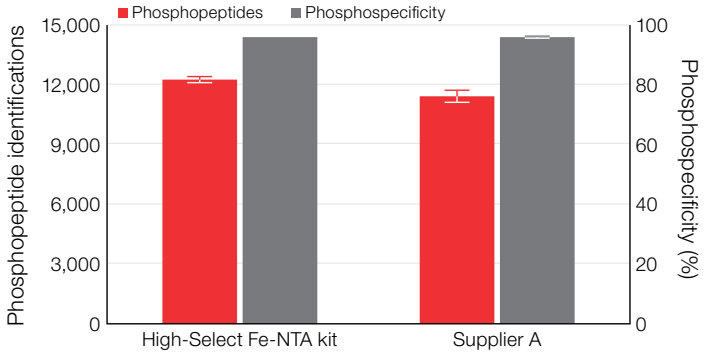


Figure 8. Phosphopeptide enrichment from nocodazole-treated HeLa cell digests. 1 mg of digest was enriched using 25 µL of Fe-NTA magnetic beads before LC-MS analysis (n = 3). Enrichment using Thermo Scientific™ High-Select™ Fe-NTA Magnetic Agarose beads had higher phosphopeptide identifications and equivalent phosphospecificity (97%) compared to another supplier's resin.

EasyPep mini, maxi, and 96-well plate MS sample prep kits

Simplified sample preparation solutions for mass spectrometry

Sample preparation of peptides for MS analysis is complex, with numerous steps and nonstandard protocols, resulting in variable sample quality and poor reproducibility. To address these issues, the Thermo Scientific™ EasyPep™ MS Sample Prep Kits have been designed using a standardized workflow to improve reproducibility while also saving hands-on and processing time.

Highlights:

- **Complete**—includes preformulated reagents, and an optimized protocol for processing up to 20 samples (mini column), 8 samples (maxi column), or one 96-well plate
- **Compatible**—reagents and protocol designed for compatibility with upstream and downstream processes, including TMT and TMTpro labeling
- **Flexible**—reagents and protocol have been verified using cells, plasma, and tissue samples for 10 µg to 2 mg samples
- **Time-saving**—sample processing has been reduced from more than 1 day to less than 4 hours, with improved results (Figures 9 and 10)

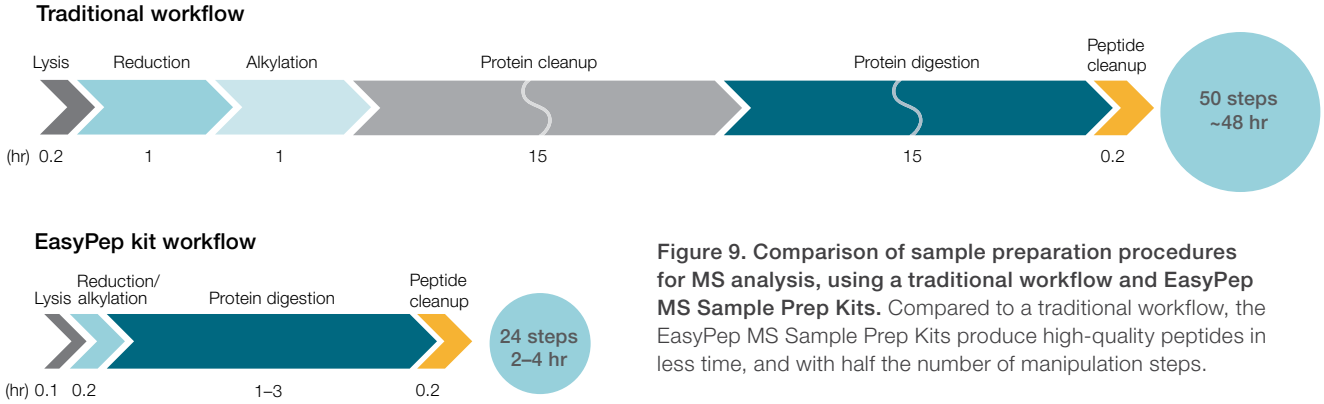


Figure 9. Comparison of sample preparation procedures for MS analysis, using a traditional workflow and EasyPep MS Sample Prep Kits. Compared to a traditional workflow, the EasyPep MS Sample Prep Kits produce high-quality peptides in less time, and with half the number of manipulation steps.

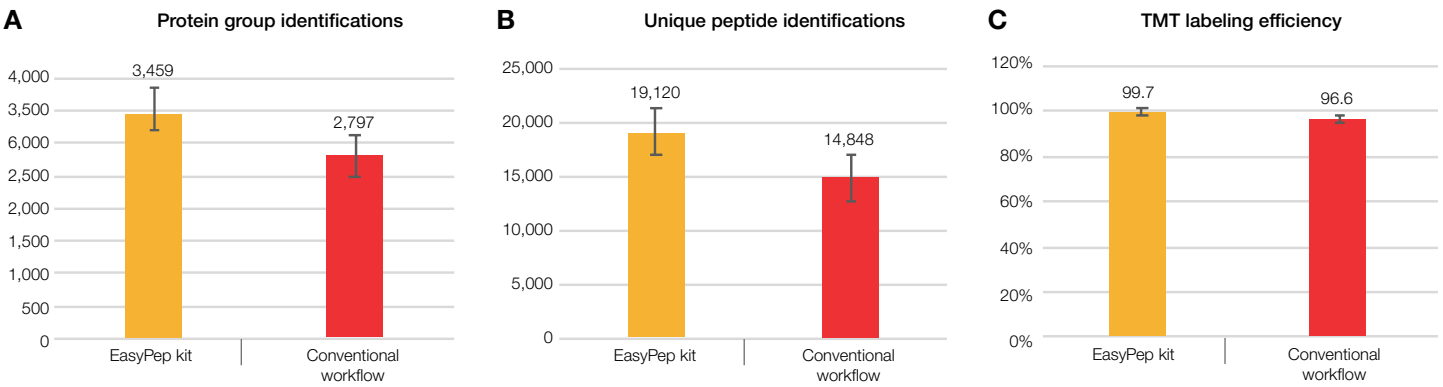


Figure 10. Comparison of the EasyPep and conventional methods for processing TMT-labeled multiplexed samples. The EasyPep kit resulted in more peptide and protein identifications with high TMT labeling efficiency (N terminus and lysine) compared to a conventional acetone precipitation workflow.

How-to video for EasyPep Mini MS Sample Prep Kit:

Use this rapid mass spec sample prep kit for protein extraction, digestion, and peptide cleanup in 2–4 hours.

Learn more at thermofisher.com/easypep

Instrument calibration

Optimized and ready-to-use controls, standards, and calibration solutions

Thermo Scientific™ Pierce™ calibration solutions for mass spectrometry are ready-to-use liquid formulations that can quickly calibrate Thermo Scientific™ LC-MS instrumentation. In addition to calibration solutions, standards for sensitivity assessment, chromatographic performance, determination of digestion efficiency, top-down method development, or as a control for sample analysis are available (Tables 4 and 5). Also available are high-purity, validated mobile phases and acidic ion-pairing agents that are essential for achieving effective and reproducible liquid chromatography (LC) separation of peptides for electrospray ionization (ESI) MS.

Highlights:

- **Optimized**—products developed to maximize LC-MS instrument performance (Figures 11 and 12)
- **Convenient**—calibration solutions, standards, and solvent blends require little or no preparation
- **High purity**—all reagents provided in nonleachable containers
- **Validated**—all products have been manufactured in an ISO 9001–certified facility and fully tested using Thermo Scientific™ mass spectrometers
- **Long shelf life**—products stable for a minimum of 1 year if stored properly

Table 4. Choose the right calibration solution for your platform.

Thermo Scientific™ calibration solution	Components	Mass range	Calibration mode	Storage	Recommended Thermo Scientific™ mass spec instrument series
Pierce FlexMix Calibration Solution for Auto-Ready Mass Spectrometers	16 components*	50–3,000	Positive and negative	Room temperature	Orbitrap IQ-X MS
Pierce FlexMix Calibration Solution	16 components*	50–3,000	Positive and negative	Room temperature	Orbitrap Fusion, Orbitrap Fusion Lumos, Orbitrap ID-X MS
Pierce LTQ ESI Positive Ion Calibration Solution	Caffeine, MRFA, Ultramark 1621	195–1,522	Positive	2–8°C	LTQ, LTQ Orbitrap, LXQ, LCQ FLEET, Exactive
Pierce LTQ Velos ESI Positive Ion Calibration Solution	Caffeine, MRFA, Ultramark 1621, *n*-butylamine	74–1,522	Positive	2–8°C	LTQ Velos/Velos Pro, LTQ Orbitrap Velos/Orbitrap Velos Pro, Orbitrap Elite, Q Exactive, Orbitrap Fusion Tribrid
Pierce ESI Negative Ion Calibration Solution	SDS, sodium taurocholate, Ultramark 1621	265–1,680	Negative	2–8°C	LTQ, LTQ Velos, LTQ Orbitrap, Exactive
Pierce Triple Quadrupole Calibration Solution	$Tyrosine_1$ $Tyrosine_3$ $Tyrosine_6$	182–997	Positive	2–8°C	TSQ Quantum, TSQ Discovery, TSQ Quantum Access, TSQ Vantage, TSQ Endura, TSQ Quantiva
Pierce Triple Quadrupole Calibration Solution, Extended Mass Range	17 components*	69–2,722 (positive) 69–2,934 (negative)	Positive and negative	2–8°C	TSQ Quantum, TSQ Discovery, TSQ Quantum Access, TSQ Vantage, TSQ Endura, TSQ Quantiva, TSQ Fortis, TSQ Quantis, TSQ Altis

* See website for details.

Table 5. Choose the right MS standard for your application.

	Pierce Reserpine Standard	Pierce Small Molecule System Suitability Standard	Pierce BSA Protein Digest	Pierce 6 Protein Digest	Pierce Yeast Digest Standard	Pierce HeLa Protein Digest Standard	Pierce HeLa Digest/PRTC Standard	Pierce TMT11plex Yeast Digest Standard
Components	Reserpine	Glycine, atenolol, flumetsulam, atrazine, terfenadine, warfarin, Ultramark 1621, methyl malonic acid, rafoxinide	BSA tryptic peptides	Lysozyme, BSA, cytochrome C, alcohol dehydrogenase, β-galactosidase, apotransferrin (multi-species)	Yeast lysate peptide digest	HeLa lysate peptide digest	HeLa digest plus PRTC peptides (Cat. No. 88320, 88321)	Yeast peptide digest labeled with TMT11plex label reagents
Format	Liquid	Liquid	Lyophilized	Lyophilized	Lyophilized	Lyophilized	Lyophilized	Lyophilized
Primary application	Instrument QC	Metabolomics system suitability standard	Protein positive control	Chromatography assessment	System suitability standard	System suitability standard	System suitability standard	System suitability standard
Complexity of standard	+	+++	++	+++	+++	++++	++++	++++
Recommended storage	4°C	4°C	–20°C	–20°C	–20°C	–20°C	–20°C	–20°C

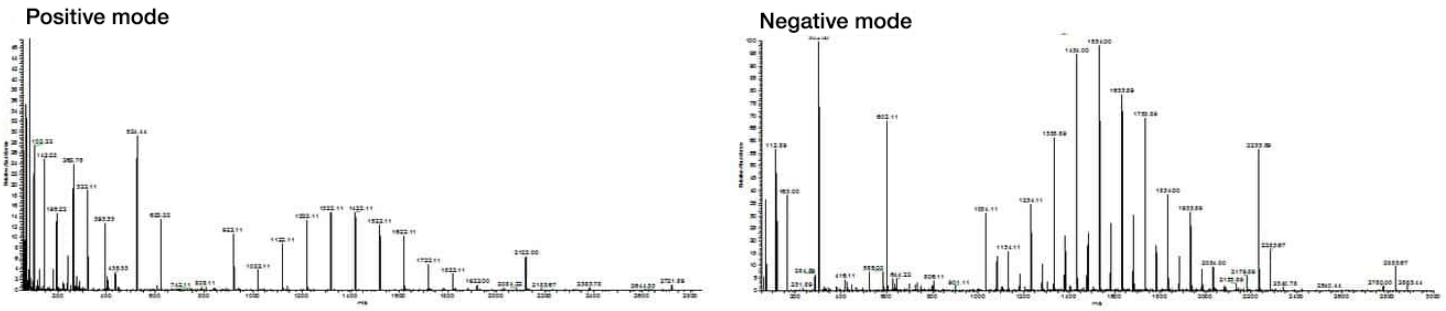


Figure 11. Pierce FlexMix Calibration Solution chromatograms. Shown are sample chromatograms in positive and negative modes.

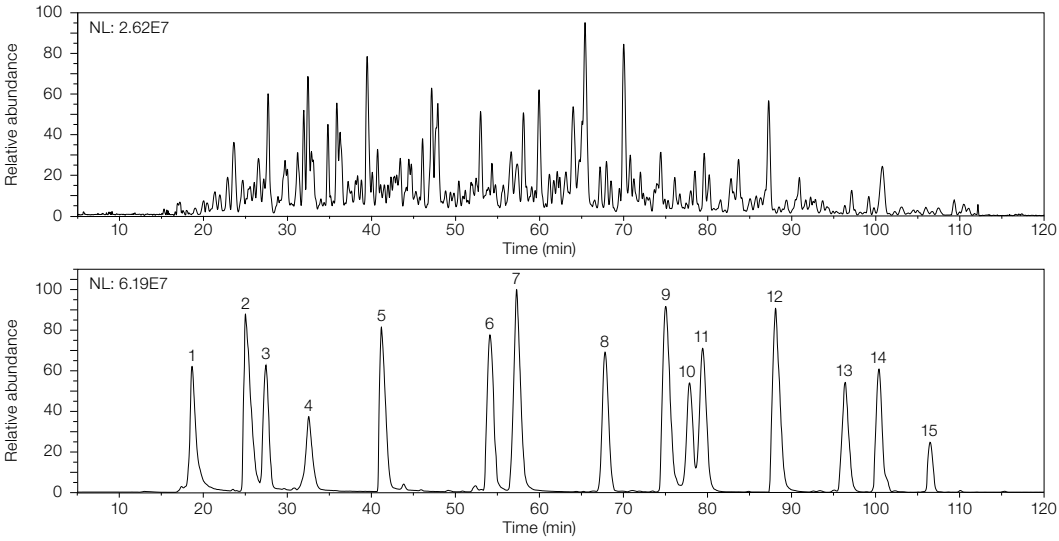


Figure 12. Example chromatograms of standard and calibration solutions. Base peak chromatogram of 200 ng Thermo Scientific™ Pierce™ HeLa Protein Digest Standard separated using a Thermo Scientific™ Acclaim™ PepMap™ 100 3 µm x 75 µm x 15 cm column with a 2–35% gradient (buffer A: 0.1% formic acid in LCMS-grade water, buffer B: 0.1% formic acid in 100% LCM-grade acetonitrile) at 300 nL/min for 120 minutes and detected on a Thermo Scientific™ LTQ Orbitrap XL™ Mass Spectrometer (top). Thermo Scientific™ Pierce™ Peptide Retention Time Calibration Mixture (10 µL at 5 pmol/µL, before dilution to 0.5 pmol/µL) was analyzed on a Thermo Scientific™ Velos Pro™ Mass Spectrometer System using a Thermo Scientific™ PepMap™ C18 column (75 µm x 15 cm) with a 2–30% gradient of buffer B using buffer A (0.1% formic acid) and buffer B (0.1% formic acid, 99.9% acetonitrile) at 0.3 µL/min (bottom).

Learn more at **thermofisher.com/mscalibration**

Test your protein research knowledge

Question: The protease of choice for mass spec sample preparation is:

A. Chymotrypsin

B. Lys-C

C. Trypsin

D. Pepsin

Answer: C

Ordering information

Product	Quantity	Cat. No.
Protein quantitation reagents		
SILAC Protein Quantitation Kit (LysC), RPMI 1640	1 kit	A33971
SILAC Protein Quantitation Kit (LysC), DMEM	1 kit	A33969
SILAC Protein Quantitation Kit (LysC), DMEM:F-12	1 kit	A33970
$^{13}C_6$ L-Arginine-HCl	50 mg	88210
$^{13}C_6$ L-Lysine-2HCl	50 mg	89988
NeuCode Lysine-080	50 mg	A33613
NeuCode Lysine-521	25 mg	A36753
NeuCode Lysine-202	25 mg	A36754
RPMI 1640 Medium for SILAC	500 mL	88365
DMEM for SILAC	500 mL	88364
TMTzero Label Reagent	5 x 0.8 mg	90067
TMTsixplex Isobaric Mass Tagging Kit	35 reactions	90064
TMTsixplex Isobaric Label Reagent Set	5 x 0.8 mg (per tag)	90066
	2 x 5 mg (per tag)	90068
TMT10plex Isobaric Label Reagent Set	1 x 0.8 mg (per tag)	90110
	3 x 0.8 mg (per tag)	90111
	1 x 5 mg (per tag)	90406
	8 x 0.2 mg (per tag)	90309
TMT11-131C Label Reagent	3 x 0.8 mg	A37724
	1 x 5 mg	A34807
TMT10plex Isobaric Label Reagent Set plus TMT11-131C Label Reagent	3 x 0.8 mg (per tag)	A37725
	1 x 5 mg (per tag)	A34808
TMTpro Zero Label Reagent	1 x 5 mg	A44518
	5 x 0.5 mg	A44519
TMTpro 16plex Label Reagent Set	1 x 5 mg (per tag)	A44520
	1 x 0.5 mg (per tag)	A44521
	6 x 0.5 mg (per tag)	A44522
TMTpro 18plex Label Reagent Set	6 x 0.5 mg	A52047
	1 x 5 mg	A52045
TMTpro-134C & TMTpro-135N Label Reagents	6 x 0.5 mg	A52048
	1 x 5 mg	A52046
Super Heavy TMT Label Reagent	2 mg	A43073
Super Heavy TMTpro Label Reagent	2 mg	A52040
IodoTMTzero Label Reagent	5 x 0.2 mg	90100
IodoTMTsixplex Label Reagent Set	5 x 0.2 mg (per tag)	90102
AminoxyTMTsixplex Label Reagent Set	5 x 0.2 mg (per tag)	90402
Sample preparation reagents		
Pierce MS-Compatible Magnetic IP Kit (Protein A/G)	40 reactions	90409
Pierce Albumin Depletion Kit	24 reactions	85160
DSSO (disuccinimidyl sulfoxide)	10 x 1 mg	A33545
DSBU (disuccinimidyl dibutyric urea)	10 x 1 mg	A35459
DSPP (disuccinimidyl phenyl phosphonic acid, PhoX)	50 mg	A52286
TBDSPP (*tert*-butyl disuccinimidyl phenyl phosphonate, tBu-PhoX)	50 mg	A52287
High-Select HSA/Immunoglobulin Depletion Mini Spin Columns	24 columns	A36366
High-Select Top14 Abundant Protein Depletion Mini Spin Columns	24 columns	A36370
Pierce Trypsin Protease, MS Grade	5 x 20 µg	90057
Pierce Trypsin Protease, MS Grade	5 x 100 µg	90058

Ordering information (cont.)

Product	Quantity	Cat. No.
Pierce LysC Protease, MS Grade	100 µg	90307
Pierce Trypsin/LysC Protease Mix, MS-Grade	100 µg	A40009
EasyPep Mini MS Sample Prep Kit	20 reactions	A40006
EasyPep 96 MS Sample Prep Kit	96 reactions	A44533
Pierce Quantitative Colorimetric Peptide Assay	500 assays	23275
Pierce Quantitative Fluorometric Peptide Assay	500 assays	23290
High-Select Fe-NTA Phosphopeptide Enrichment Kit	30 reactions	A32992
High-Select TiO_2 Phosphopeptide Enrichment Kit	24 reactions	A32993
High-Select Fe-NTA Magnetic Phosphopeptide Enrichment Kit	20 reactions	A52283
Pierce High pH Reversed-Phase Peptide Fractionation Kit	12 reactions	84868
Pierce Kinase Enrichment Kit with ATP Probe	16 reactions	88310
Pierce Detergent Removal Spin Column, 0.5 mL	25 columns	87777
Pierce Peptide Desalting Spin Columns	25 columns	89852
Pierce C18 Spin Columns	25 columns	89870
Pierce C18 Spin Tips	96 tips	84850
Calibration solutions and standards		
Pierce LTQ ESI Positive Ion Calibration Solution	10 mL	88322
Pierce LTQ Velos ESI Positive Ion Calibration Solution	10 mL	88323
Pierce ESI Negative Ion Calibration Solution	10 mL	88324
Pierce Triple Quadrupole Calibration Solution, Extended Mass Range	10 mL	88340
Pierce Reserpine Standard for LC-MS	5 x 1 mL	88326
Pierce FlexMix Calibration Solution	10 mL	A39239
Pierce Peptide Retention Time Calibration Mixture, 5 pmol/µL	200 µL	88321
Pierce BSA Protein Digest Standard, LC-MS Grade	1 nmol	88341
Pierce 6 Protein Digest Standard, Equimolar, LC-MS Grade	100 pmol	88342
Pierce Intact Protein Standard Mix	5 x 76 µg	A33527
Pierce HeLa Protein Digest Standard	5 x 20 µg	88329
Pierce HeLa Digest/PRTC Standard	10 µg	A47996
Pierce TMT11plex Yeast Digest Standard	20 µg	A40938
Pierce Small Molecule System Suitability Standard	0.25 mL	A51740

Custom peptide synthesis services

Thermo Scientific™ custom peptide synthesis services are offered for peptides of 6–70 amino acids, with a synthesis scale of 0.1 mg to 1 g, and with purity from crude to 98%.

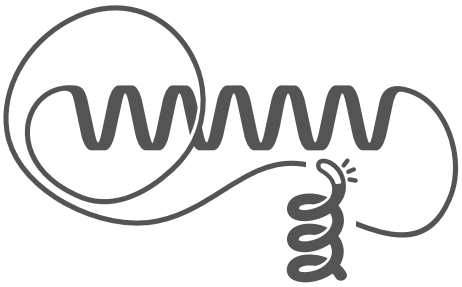
- Standard Peptide Custom Synthesis Service
- HeavyPeptide™ AQUA Custom Synthesis Service
- PEPotec™ Immuno Custom Peptide Libraries
- PEPotec™ SRM Custom Peptide Libraries

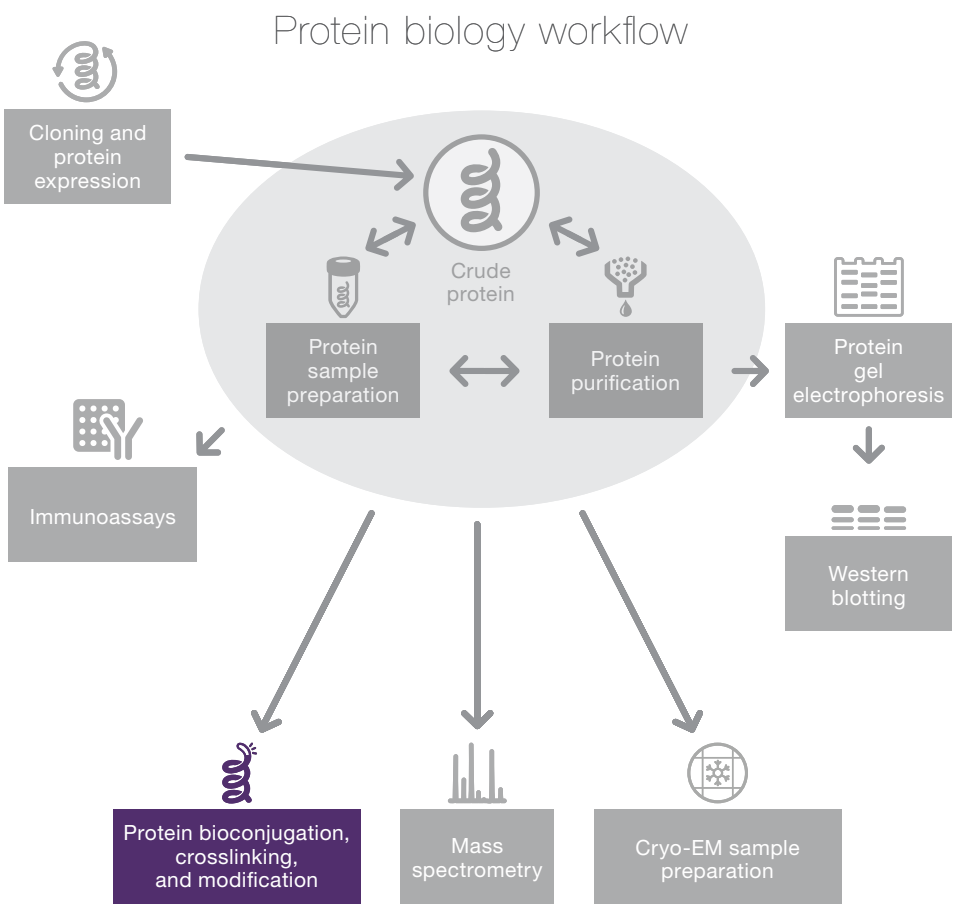
thermofisher.com/peptides

For more information, or to view additional products, go to thermofisher.com/msreagents

Protein bioconjugation, crosslinking, and modification

A number of techniques for studying the structure and interaction of proteins, as well as for manipulating proteins for use in affinity purification or detection procedures, depend on methods for chemically crosslinking, modifying, or labeling proteins and antibodies. The entire set of crosslinking and modification methods for use with proteins is also referred to as bioconjugation.





Overview

Bioconjugation is the process of chemically joining two or more molecules by a covalent bond where at least one molecule is a biomolecule. This technique utilizes a variety of reagents that contain reactive ends to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other biomolecules. The availability of several chemical groups in proteins and peptides make them targets for a wide range of applications including biotinylation, fluorescence dye conjugation, immobilization to solid supports, protein structural studies using crosslinkers, and others. Chemical agents may be used to modify amino acid side chains on proteins and peptides in order to alter charges, block or expose reactive binding sites, inactivate functions, or change functional groups to create targets for crosslinking and labeling. Bioconjugation reagents, crosslinkers, and modification reagents can be described by their chemical reactivity, molecular properties, or applications.

Chemical reactivity of bioconjugation and crosslinking reagents

The most important property of a bioconjugation reagent or crosslinker is its reactive chemical group(s). The reactive group establishes the method and mechanism for chemical modification, labeling, or crosslinking. Labeling reagents have a reactive moiety at one terminus, such as an NHS ester for amine labeling, and a chemical moiety at the other, such as a fluorescent dye or biotin, for downstream applications. Crosslinkers contain at least two reactive groups, which target common functional groups found in biomolecules such as proteins and nucleic acids. The functional groups that are commonly targeted for bioconjugation include primary amines, sulfhydryl, carbonyl, and carboxyl groups, and carbohydrates. In addition to targeting functional groups found in biomolecules, bioconjugation reagents and crosslinkers can also employ bioorthogonal groups such as azides and alkynes for click chemistry applications or photoreactive groups for nonselective labeling.

Applications

Bioconjugation and crosslinking reagents have a variety of applications in life science research and assay development. These include fluorescent labeling of proteins and peptides, protein immobilization onto solid supports, protein–protein conjugation, creation of immunotoxins, and crosslinking for protein structure and interaction studies. Protein conjugates are often designed to enable purification and detection in complex biological samples. Commonly, antibodies are the target of bioconjugation with common labels such as biotin or chemically reactive fluorescent dyes. In this process, NHS ester chemistry is the most widely used method for labeling available lysine residues.

Key considerations

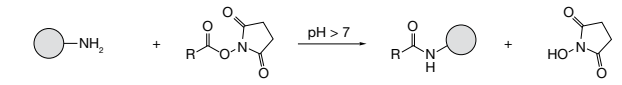
Bioconjugation and crosslinking reagents are selected based on their chemical reactivities and other chemical properties that affect their behavior in different applications. Key considerations include the chemical specificity of the reactive ends, reaction conditions, and whether further modification to the protein or peptide of interest is required to enable bioconjugation. Other important factors that influence the functionality, specificity, and solubility of the bioconjugation reaction include the spacer arm length, cleavability, composition, and structure (Table 1).

Table 1. Key considerations for selecting the right bioconjugation reagent.

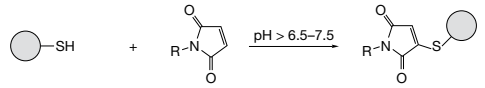
Chemical reactivity

First, select a reagent with the functional group(s) to bind your biomolecules of interest.

NHS ester reaction



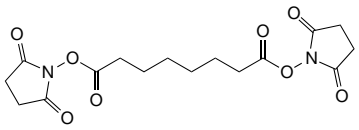
Maleimide reaction



Molecular properties

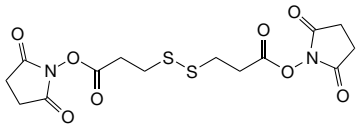
Second, choose which features or characteristics are important for your application.

Functionality



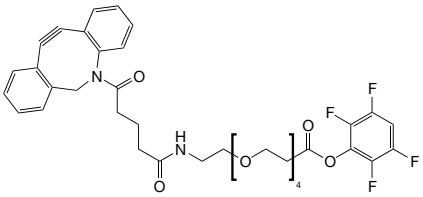
DSS—same reactivity on both ends

Cleavability



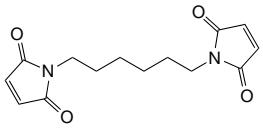
DSP with disulfide linker for cleavage

Structural modifications



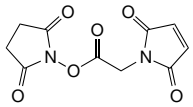
DBCO—copper-free click moiety

Spacer arm composition



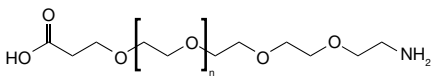
BMH with hydrocarbon spacer

Spacer arm length



AMAS 4.4 Å—short spacer between reactive groups

Spacer arm structure

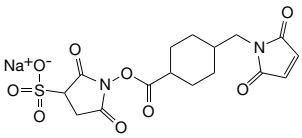


CA(PEG)$_n$—adds solubility in aqueous solutions

Applications

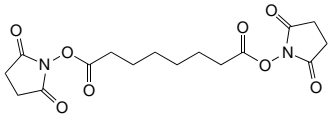
Select the specific reagent depending on the application (e.g., protein detection, immobilization, or interaction studies).

Conjugation



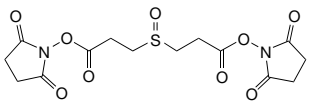
Sulfo-SMCC

Immobilization



DSS—couples proteins to surfaces

Protein interaction studies



DSSO—MS-cleavable crosslinker

Packaging options

Select a package size or grade based on the scale of your reaction or your requirements. These reagents are available from milligram to kilogram quantities.

Milligram

Single tubes

Milligram to gram

Catalog product

Milligram to gram

Premium-grade

Gram to kilogram

Large-volume or custom packages

Protein crosslinking

Our crosslinking reagents are designed for a variety of techniques including protein–protein conjugation, protein immobilization, antibody–drug conjugation, and protein structural studies. We offer a complete portfolio of crosslinkers tailored to a variety of applications with homobifunctional crosslinkers having the same reactive chemistry at both ends and heterobifunctional crosslinkers having different chemistries at each end. Choosing the right crosslinker depends on the chemical specificity of the reactive ends, reaction conditions, and spacer composition. Depending on the application, it may be desirable to adjust the degree of protein labeling (Figure 1).

Highlights:

- **Wide selection**—crosslinkers can target a wide variety of functional groups for tailored conjugation on proteins (amines and sulfhydryls) and bioorthogonal reactivity (Table 2)
- **Diverse composition**—choose from multiple spacer arm lengths, hydrophobicity, and cleavability
- **Multiple formats**—bulk, Thermo Scientific™ No-Weigh™ format, and optimized kit packaging available

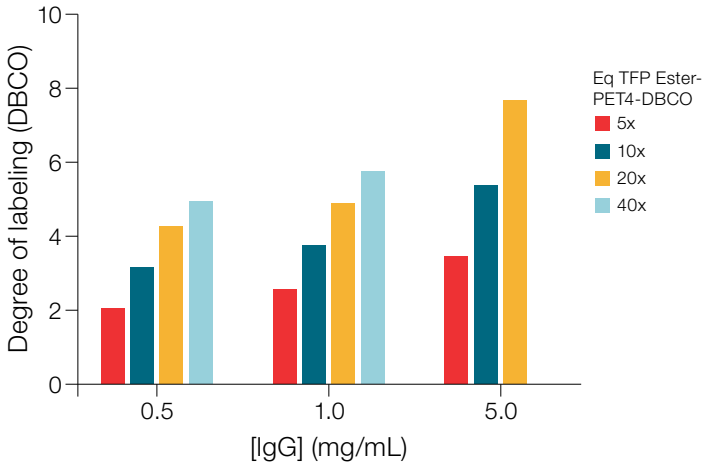


Figure 1. The level of DBCO labeling of antibodies or other proteins can be controlled by varying the concentration and ratio of protein to TFP Ester-PEG_4-DBCO using the Thermo Scientific™ EZ-Link™ DBCO Protein Labeling Kit.

Table 2. Overview of crosslinker reactivities and examples.

Reactivity class	Target functional group	Reactive chemical group	Examples
Amine-reactive	NH_2	NHS ester	NHS, sulfo-NHS
		Imidoester	DMS, DMP
Carboxyl- and amine–reactive	COOH	Carbodiimide	EDC, DCC
Sulfhydryl-reactive	SH	Maleimide	SMCC, sulfo-SMCC
		Haloacetyl	SIAB, sulfo-SIAB, SIA
		Piridyl disulfide	PDPH
Aldehyde-reactive	CHO	Hydrazide	BMPH, EMCH, PMPH
Photoreactive	Random	Diazirine	SDA, sulfo-SDA
		Aryl azide	Sulfo-SANPAH
Hydroxyl (nonaqueous)-reactive	OH	Isocyanate	PMPI
Azide-reactive	N_3	Phosphine	NHS-azide, NHS-PEG_n-azide
		DBCO/alkyne	

Learn more at **thermofisher.com/proteincrosslinking**

Protein modification

Protein analysis and detection techniques often require more than direct conjugation with a bifunctional crosslinker or activated labeling reagent. For example, in many situations, specialized protein modifications are needed to add molecular mass, increase solubility for storage, or create a new functional group that can be targeted in a subsequent reaction step. Our extensive portfolio of modification reagents enables you to modify your protein for your specific experimental need (Table 3).

Highlights:

- **Convenient**—ready-to-use reagents and convenient single-use packaging available
- **Broad portfolio**—extensive offering of reagents to increase solubility, block or derivatize functional groups, and reduce proteins
- **High quality**—products manufactured in ISO 9001–certified facilities

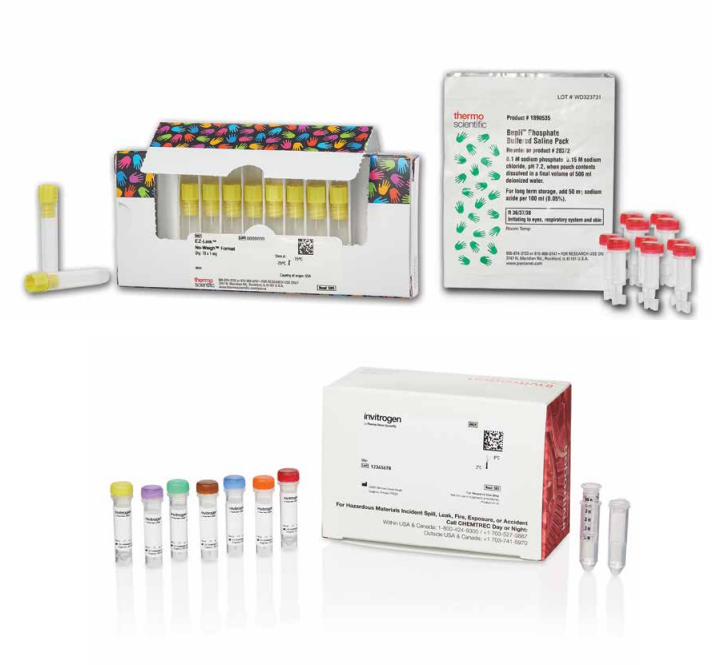
Table 3. Overview of key protein modification reagents.

Reagent	Reactive groups	Application
2-Mercaptoethanol	Thiol	Reducing agent
Sodium cyanoborohydride	Cyanoborohydride	Reduce Schiff base to alkylamine linkage
CA(PEG)$_n$	Amine Carboxylic acid	Reversibly block primary amines (CA(PEG)$_4$); PEGylation of protein or surface, terminating with a carboxylic acid or primary amine
Citrionic anhydride	NA	Reversibly block primary amines
CT(PEG)$_{12}$	Carboxylic acid Thiol	PEGylation of gold or metal surfaces, terminating with a carboxylic acid
Cysteine HCl	Thiol	Reducing agent
DTT, Cleland's reagent	Thiol	Reducing agent
Ethylenediamine 2HCl	Amine	Addition of amines to protein or surface
Guanidine HCl	NA	Protein denaturant
Hydroxylamine	NA	Deprotecting SATA-modified molecules
Iodoacetic acid	Iodoacetyl	*S*-carboxymethylation of sulfhydryls (reduced cysteines)
MA(PEG)$_n$	Amine	PEGylation of a protein, oxidized carbohydrate, or surface terminating with a methyl group
MM(PEG)$_{12}$, MM(PEG)$_{24}$	Maleimide	Branched PEGylation of protein or surface, terminating with methyl group
MS(PEG)$_n$	*N*-hydroxysuccinimide ester	PEGylation of a protein or surface, terminating with methyl group
NEM	Maleimide	Irreversible blocking of sulfhydryl groups
SATA	*N*-hydroxysuccinimide ester	Modification of primary amines with protected sulfhydryl
Sodium meta-periodate	Periodate	Oxidize carbohydrates for reductive amination
Sodium NHS-acetate	*N*-hydroxysuccinimide ester	Irreversibly block amines
TCEP	Phosphine	Reducing agent
Traut's reagent	Iminothiolane	Modify primary amines to contain a free sulfhydryl group
Urea	NA	Reducing agent

Learn more at **thermofisher.com/proteinmodification**

Biotinylation

The highly specific interaction of avidin with biotin is a valuable tool in designing nonradioactive purification and detection systems. The extensive line of Thermo Scientific™ biotinylation labeling reagents are designed to be conjugated to an antibody, protein, peptide, cell surface proteins, or nucleic acids (Table 4). Multiple types of biotinylation reagents are available with varied uses preferable for specific applications, so we've developed easy-to-use guides for selecting the right reagent. In addition to bulk packaging, we offer the easy-to-use No-Weigh format and optimized kits of our biotinylation reagents.



Highlights:

- **Multiple reactivities**—wide selection of amine-, sulfhydryl-, carbonyl-, UV-, and click-reactive biotinylation reagents
- **Reversible and cleavable**—desthiobiotin and cleavable biotin for capture and release applications
- **Application-specific**—reagents designed for specific applications such as cell surface protein labeling or site-specific antibody labeling

Table 4. Recommended biotinylation reagents for specific targets and applications.

Target	Example	Reactivity	Application
Antibody	Sulfo-NHS-LC-biotin	Amine-reactive	ELISA, blotting, imaging
	Biotin-PEG_4-hydrazide	Carbohydrate-reactive	
	SiteClick Biotin Antibody Labeling Kit	Azide- and alkyne-reactive	
Purified protein	Sulfo-NHS-SS-biotin	Amine-reactive	ELISA, blotting, or affinity purification of receptor or antigen
	Maleimide-PEG_2-biotin	Sulfhydryl-reactive	
	Biotin-PEG_4-hydrazide	Carbohydrate-reactive	
Purified peptide	NHS-PEG_4-biotin	Amine-reactive	ELISA, blotting, or affinity purification of receptor or antigen
	Maleimide-PEG_2-biotin	Sulfhydryl-reactive	
	Amine-PEG_2-biotin	Carbohydrate- and amine-reactive	
Cell surface proteins	Cell Surface Protein Isolation Kit	Amine-reactive	Affinity purification or removal of cell surface receptor ligands
DNA/RNA or oligonucleotides	Psoralen-PEG_3-biotin	Random	Capture or detection of oligonucleotides in ELISA-type applications or affinity purification
	Amine-PEG_2-biotin	Carbohydrate- and amine-reactive	

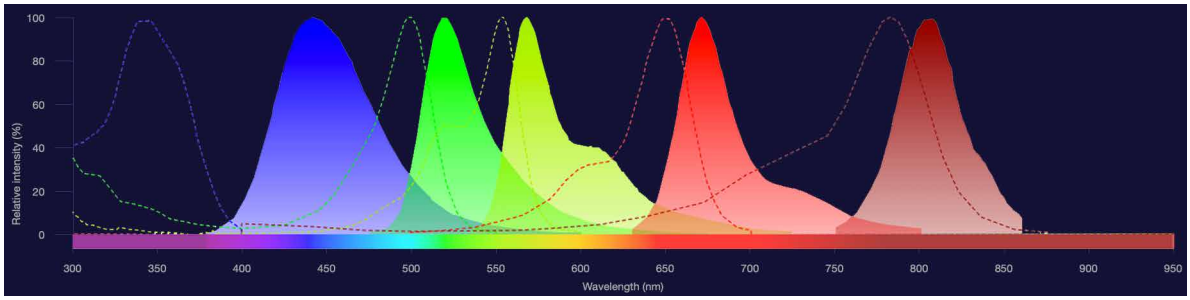
Learn more at **thermofisher.com/biotinlabeling**

Fluorophore labeling

Fluorophores are invaluable tools for detecting protein location and activation, identifying protein complex formation and conformational changes, and monitoring biological processes *in vivo*. The vast selection of fluorophores today provides greater flexibility, variation, and fluorophore performance for research applications than ever before. Our large selection of superior Invitrogen™ Alexa Fluor™ dyes across the spectrum enables labeling of a variety of molecules including peptides, proteins, and antibodies. Dyes are compatible with fluorescence microscopy, flow cytometry, high-content analysis, high-density arrays, *in vivo* imaging, immunoblotting, and immunostaining applications.

Highlights:

- **Superior brightness**—conjugates made with Alexa Fluor dyes produce fluorescence output that surpasses that of spectrally similar fluorophore-labeled conjugates
- **Excellent photostability**—lower photobleaching rates allow for more time for image capture
- **Across the spectrum**—dyes span from the near-UV, visible, and near-IR spectrum



Learn more at **thermofisher.com/fluorescentlabeling**

Special packaging options

Optimized kits

We've packaged many of our popular fluorophores, biotinylation reagents, and crosslinkers in easy-to-use optimized kits for labeling and crosslinking. Each kit contains everything needed to label a protein or antibody with the desired label including the reagents, conjugation buffer, purification system, and an easy-to-follow, step-by-step protocol for conjugation.

Highlights:

- **Scalable**—kits are designed for labeling as little as 100 µg of protein and up to 10 mg
- **Easy**—everything included with an optimized workflow for labeling
- **Wide selection**—kits are available for labeling with Alexa Fluor dyes, biotin, and crosslinkers

Learn more at **thermofisher.com/labelingkits**

No-Weigh packaging format

Bioconjugation reagents are highly reactive molecules and therefore may be sensitive to water, light, oxygen, or other surrounding conditions. They may form unstable intermediates that lead to undesired side products of the intended reactions. Therefore, it is critical to minimize the exposure of bioconjugation reagents to these negative environmental factors to obtain the highest yields and quality possible.

To mitigate these risks, we offer specific packaging and quality grades for your bioconjugation reagents. In addition to these options, our bioconjugation reagents are available in multiple pack sizes. Custom packaging and pack sizes are also available by request.

With the convenient Thermo Scientific™ No-Weigh™ format, a ready-to-use solution can be made quickly and conveniently. This unique packaging format helps to eliminate the need to weigh out small volumes of dry chemicals. Once reconstituted, the reagent is ready to use at the desired concentration. Avoid weighing hassles and wasting precious reagents with our single-use No-Weigh packaging format.

Highlights:

- **Helps save time**—avoid weighing chemicals; just add water, buffer, or solvent to create a working solution in seconds
- **Helps reduce waste**—small working aliquots limit the amount of unused material discarded
- **Always fresh**—working solution is ready to use at desired concentration; no need to store stock solutions

Learn more at **thermofisher.com/no-weigh**

Pierce Premium Grade reagents

Thermo Scientific™ Pierce™ Premium Grade reagents are an ideal choice for applications where product integrity and risk minimization are critical. Compared to standard-grade reagents, Pierce Premium Grade reagents provide clearly defined quality by including batch-specific information such as quality assurance review, lot sample retention, and change control notification (CCN), as well as an enhanced level of analytical testing and product characterization.

Highlights:

- **Quality reagents**—high-purity reagents that can be used to create high-quality activated derivatives, labeled proteins, and bioconjugates
- **Product integrity**—enhanced level of testing and characterization
- **Lot retention**—ample supply of past lots retained to ensure future process testing

Learn more at **thermofisher.com/premium-grade**

Test your protein research knowledge

Question: What kind of bond is involved in the bioconjugation of two molecules?

A. Hydrogen

B. Covalent

C. Cohesive

D. Ionic

Answer: B

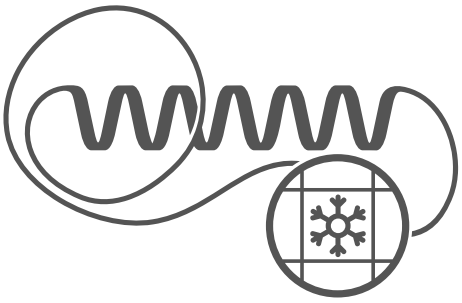
Ordering information

Product	Quantity	Cat. No.
Crosslinkers		
NHS (*N*-hydroxysuccinimide)	25 mg	24500
Sulfo-NHS (*N*-hydroxysulfosuccinimide)	500 mg	24510
Pierce EDC, No-Weigh Format	10 x 1 mg	A35391
EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride)	25 g	22981
BS3 (bis(sulfosuccinimidyl)suberate)	50 mg	21580
SPDP (*N*-succinimidyl 3-(2-pyridyldithio) propionate)	50 mg	21857
Pierce SMCC, No-Weigh Format	10 x 1 mg	A35394
Sulfo-SMCC (sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate)	50 mg	22322
SMPH (succinimidyl 6-((β-maleimidopropionamido) hexanoate))	50 mg	22363
Pierce DSG, No-Weigh Format	10 x 1 mg	A35392
DSG (disuccinimidyl glutarate)	50 mg	20593
Pierce DSP, No-Weigh Format	10 x 1 mg	A35393
DTSSP (3,3′-dithiobis(sulfosuccinimidyl propionate))	50 mg	21578
DMS (dimethyl suberimidate)	1 g	20700
DMP (dimethyl pimelimidate)	1 g	21667
N,*N*′-dicyclohexylcarbodiimide	100 g	113901000
Sulfo-SIAB (sulfosuccinimidyl (4-iodoacetyl)aminobenzoate)	50 mg	22327
SIA (succinimidyl iodoacetate)	50 mg	22349
PDPH (3-(2-pyridyldithio)propionyl hydrazide)	50 mg	22301
BMPH (*N*-β-maleimidopropionic acid hydrazide)	50 mg	22297
Sulfo-SDA (sulfo-NHS-diazirine) (sulfosuccinimidyl 4,4′-azipentanoate)	50 mg	26173
Sulfo-SANPAH (sulfosuccinimidyl 6-(4′-azido-2′-nitrophenylamino)hexanoate)	50 mg	22589
PMPI (*N*-(*p*-maleimidophenyl) isocyanate)	50 mg	28100
NHS-PEG_4-Azide	100 mg	26130
EZ-Link TFP Ester-PEG_4-DBCO	25 mg	C20039
View all available products at **thermofisher.com/crosslinking**		
Protein modification reagents		
Pierce 2-Mercaptoethanol	10 x 1 mL	35602BID
AminoLink Reductant (sodium cyanoborohydride)	2 x 1 g	44892
$CA(PEG)_4$	100 mg	26120
Citraconic Anhydride	25 g	154790250
$CT(PEG)_{12}$	100 mg	26133
Pierce Cysteine-HCl	5 g	44889
Pierce DTT, Cleland's Reagent	5 g	20290
Guanidine-HCl	500 g	24110
Pierce Hydroxylamine-HCl	25 g	26103
Pierce Iodoacetic Acid	500 mg	35603
Pierce $BS(PEG)_5$, No-Weigh Format	10 x 1 mg	A35396
Pierce $SM(PEG)_2$, No-Weigh Format	10 x 1 mg	A35397
Pierce $SM(PEG)_{12}$, No-Weigh Format	10 x 1 mg	A35398
$MM(PEG)_{12}$	100 mg	22711
$MS(PEG)_4$	100 mg	22341
$MT(PEG)_4$	100 mg	26132
Pierce NEM (*N*-ethylmaleimide)	25 g	23030
Pierce SATA (*N*-succinimidyl S-acetylthioacetate)	50 mg	26102

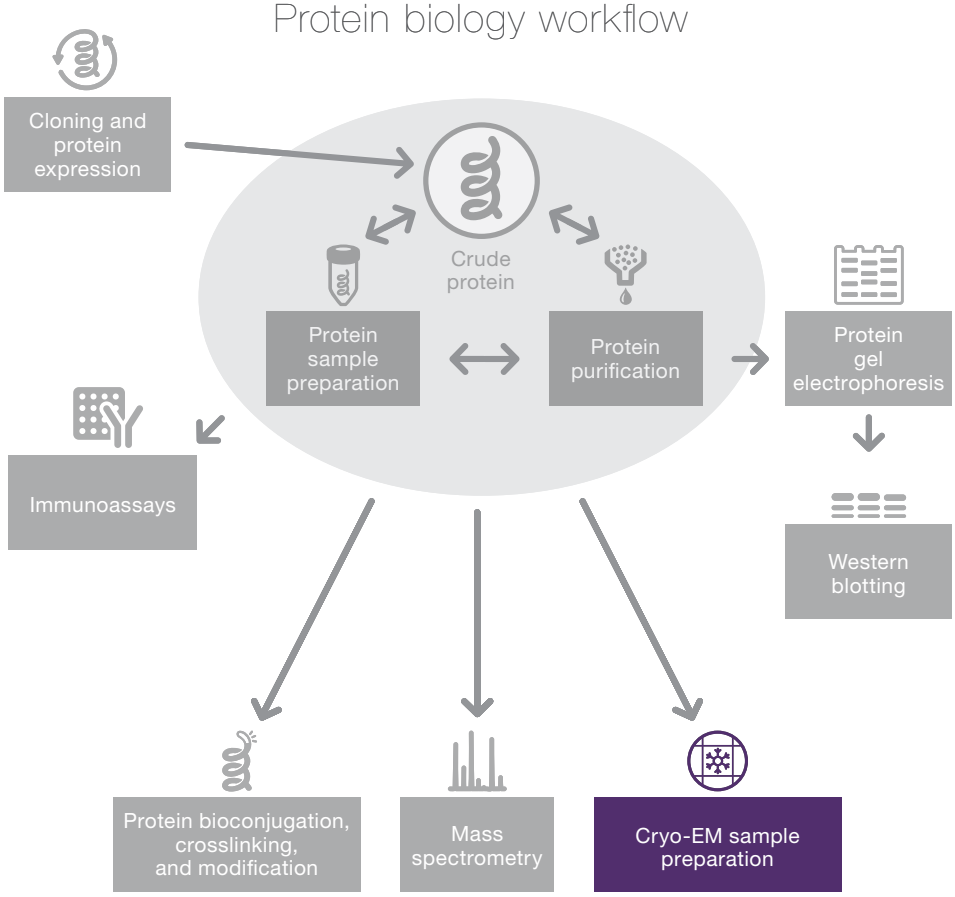
Product	Quantity	Cat. No.
Pierce TCEP-HCl, No-Weigh Format	10 x 1 mg	A35349
Bond-Breaker TCEP Solution, Neutral pH	5 mL	77720
Pierce Traut's Reagent (2-iminothiolane HCl)	500 mg	26101
Urea	1 kg	29700
View all available products at **thermofisher.com/proteinmodification**		
Biotinylation reagents		
EZ-Link NHS-PEG_{12}-Biotin, No-Weigh Format	10 x 1 mg	A35389
EZ-Link NHS-PEG_{12}-Biotin	500 mg	21313
EZ-Link Sulfo-NHS-LC-Biotin	100 mg	21335
EZ-Link Sulfo-NHS-LC-LC-Biotin, No-Weigh Format	10 x 1 mg	A35358
EZ-Link Sulfo-NHS-LC-LC-Biotin	50 mg	21338
EZ-Link Sulfo-NHS-SS-Biotin	100 mg	21331
EZ-Link NHS-LC-Biotin	50 mg	21336
EZ-Link Maleimide-PEG_2-Biotin	50 mg	21901BID
EZ-Link Amine-PEG_3-Biotin	50 mg	21347
EZ-Link NHS-PEG_4-Biotin	50 mg	21362
EZ-Link HPDP-Biotin	50 mg	21341
SiteClick Biotin Antibody Labeling Kit	1 kit	S20033
View all available products at **thermofisher.com/biotinlabeling**		
Fluorescent reactive dyes		
Alexa Fluor 350 NHS Ester	5 mg	A10168
Alexa Fluor 488 NHS Ester	5 mg	A20100
Alexa Fluor 532 NHS Ester	5 mg	A20101MP
Alexa Fluor 546 NHS Ester	5 mg	A20102
Alexa Fluor 555 NHS Ester	5 mg	A20109
Alexa Fluor 568 NHS Ester	5 mg	A20103
Alexa Fluor 594 NHS Ester	5 mg	A20104
Alexa Fluor 633 NHS Ester	5 mg	A20105
Alexa Fluor 647 NHS Ester	5 mg	A20106
Alexa Fluor 660 NHS Ester	1 mg	A20007
Alexa Fluor 700 NHS Ester	5 mg	A20110
Alexa Fluor 750 NHS Ester	5 mg	A20111
View all available products at **thermofisher.com/fluorescentlabeling**		

Cryo-EM sample preparation

Obtaining a protein structure is critical to understanding protein function. In recent years cryo-electron microscopy (cryo-EM) has seen rapid adoption among structural biologists due to its ability to image even the most difficult targets, like membrane proteins and complex molecular machinery, as well as the achievable resolution that is getting ever closer to matching that of X-ray crystallography.



Protein biology workflow



Cryo-EM techniques

Cryo-EM cools samples to cryogenic temperatures so quickly that it prevents water molecules from crystalizing, preserving the native sample structure. Once frozen, a range of EM techniques can be used to visualize the specimen in 3D at a variety of resolutions—including near-atomic resolution—allowing for deeper, more comprehensive insights than previously possible.

SPA

Single particle analysis (SPA) enables near-atomic structural determination of challenging proteins and protein complexes without the need for crystallization. In SPA, purified proteins or protein complexes are suspended in amorphous (vitreous) ice through rapid plunge freezing, which preserves the native structures of the samples. Transmission electron microscopy (TEM) is then used to collect numerous 2D snapshots of the samples. As the proteins are oriented randomly within the ice, these images show the sample at various angles, and can be recombined into a high-resolution 3D reconstruction of the sample.

MicroED

Microcrystal electron diffraction (MicroED) enables fast, high-resolution, structural determination of small molecules and proteins. Atomic details can be extracted from individual nanocrystals (<200 nm in size), even in a heterogeneous mixture. Data is acquired by cryo-TEM, using electrons as the incident beam. Since MicroED is a diffraction technique, samples need to be crystallized using the same methods that are found in X-ray crystallography. However, much smaller crystals (~100 nm in size) can be used in MicroED because the interaction of the crystal with electrons is much stronger than its interaction with X-rays. This may significantly shorten the sample preparation process and allow for the analysis of crystals that are too small to diffract with other methods.

Cryo-ET

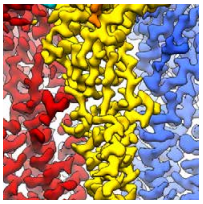
Cryo-electron tomography (cryo-ET) provides label-free, fixation-free, nanometer-scale imaging of a cell's interior in 3D and visualizes protein complexes within their physiological environments. Using a correlative light and electron microscopy approach allows targeting of tagged proteins by fluorescence microscopy before subsequent cryo-EM higher-resolution imaging. Many cells are too thick for electrons, so the vitrified cells must be thinned with a cryo-focused ion beam (cryo-FIB) microscope prior to imaging in a transmission electron microscope.

Protein sample preparation for cryo-EM analysis

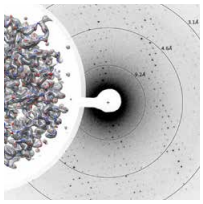
The most commonly used cryo-EM method today is SPA, in which purified proteins are imaged to high-resolution 3D structures. SPA relies on near instantaneous freezing of proteins from solution to a thin layer of noncrystalline ice on a grid in a process called vitrification. This process allows for capturing the protein in its native state and providing a protective environment for subsequent electron-based imaging at high vacuum in the transmission electron microscope.

Sample preparation is currently a major bottleneck in SPA. Even though semiautomated vitrification robots like the Thermo Scientific™ Vitrobot™ Mark IV System help to make parts of the grid preparation more robust, the process can still prove challenging as many different parameters contribute to the quality of the prepared grid. When beginning cryo-EM sample preparation, it is critical to have a well-characterized and homogeneous sample, preferably tested by negative stain imaging or mass spectrometry. Nevertheless, even purified proteins that are stable and structurally intact can behave differently in a thin vitreous ice layer, exhibiting unwanted behavior such as denaturation, aggregation, or preferred orientation. Due to the unique properties of each protein, multiple rounds of optimization are often necessary, where vitrification parameters, grid types, or additives are adjusted before the optimal condition for high-resolution data collection are found (i.e., particles are structurally intact, randomly oriented, and equally distributed). Currently, sample optimization is often performed in a nonsystematic way, extending optimization time over multiple days or even weeks.

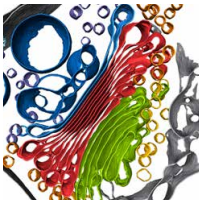
In order to support the successful preparation of cryo-EM samples, we offer solutions for every step of the cryo-EM workflow to help to train new users, optimize sample conditions, and provide standards that can be used for benchmarking or workflow optimization (Figure 1).



Single-particle analysis



MicroED



Cryo-ET

Protein expression
- Mammalian cell expression
- Cell-free expression

Cell growth

Protein extraction and isolation
- Cell lysis
- Detergents for protein solubilization

Protein purification
- Recombinant protein purification
- Biotin affinity purification
- NanoDrop spectrophotometers

Protein clean-up
- Zeba spin desalting columns
- Protein concentrators

Cryo-EM standards and training
- VitroEase Apoferritin Standard
- VitroEase Training Kit

Crystal growth

EM sample optimization
- VitroEase Buffer Screening Kit
- EM-compatible detergents

Grid selection

Negative stain screening
- VitroEase Methylamine Vanadate
- VitroEase Methylamine Tungstate

Vitrification
- Vitrobot Mark IV System

Lamellae preparation
- Cryo-FIBs

Cryo-EM
- Tundra Cryo-TEM

- Krios G4 Cryo-TEM
- Glacios 2 Cryo-TEM

Figure 1. Sample preparation workflows for high-resolution cryo-EM.

Cryo-EM sample preparation products

VitroEase Cryo-EM Training Kit and Apoferritin Standard

The Thermo Scientific™ VitroEase™ Cryo-EM Training Kit and Apoferritin Standard provide a complete solution for the successful preparation of grids for cryo-EM SPA.

Highlights:

- **Positive control sample**—highly pure apoferritin protein verified for use in cryo-EM grid preparation and analysis
- **High-quality**—grids and accessories from trusted sources
- **Convenient**—ready-to-use protein standard with step-by-step instructions for successful grid preparation

The VitroEase Cryo-EM Training Kit has been designed to provide the necessary components to get started with cryo-EM analysis of protein samples. The kit includes a positive control protein and commonly used accessories used in grid preparation, including tweezers for manipulation, grids, and grid boxes for storage. Detailed step-by-step instructions guide the user through the lengthy vitrification process.

The VitroEase Apoferritin Standard has been designed specifically to meet the stringent needs for high protein purity, minimal protein aggregation, and intact protein structure. Apoferritin, a 24-subunit protein (474 kDa), is considered the gold standard for cryo-EM SPA (Figure 2). The standard is available in the kit and sold separately.

Together, the control and components may be used as a comparison for success for new users or for quality control for experienced users.

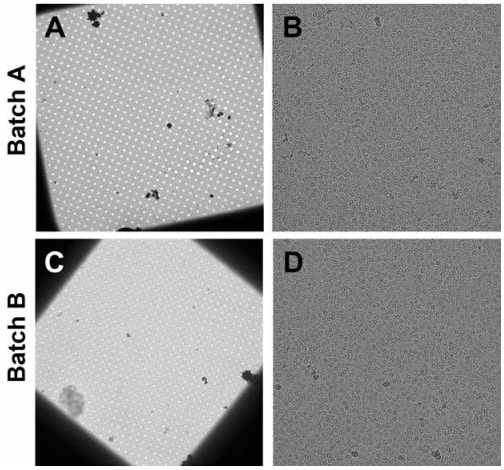


Figure 2. Representative micrographs at (A,C) grid square level and (B,D) hole level of two different VitroEase Apoferritin Standard batches prepared with the recommended vitrification settings.

VitroEase Buffer Screening Kit

The Thermo Scientific™ VitroEase™ Buffer Screening Kit provides preformulated buffers and detergents with a comprehensive screening strategy to identify optimal protein conditions for cryo-EM SPA.

Highlights:

- **Ease of use**—preformulated buffers and detergents at 10X concentration
- **Comprehensive**—14 buffers and 6 detergents commonly used for cryo-EM sample preparation provided with an optimized screening strategy
- **Flexible**—range of salts, pH, and detergent formulations for sample optimization of 400 grids
- **Validated**—functionally validated using cryo-EM SPA

Cryo-EM sample screening evaluates protein behavior in various buffers and additive conditions with the end goal of identifying the optimal conditions in which the protein is stable, randomly oriented, and equally distributed in ice. Kit buffers are provided with a pH range from 3.6 to 8.9, premixed with various salts and divalent cations.

The comprehensive sample screening strategy uses two rounds of screening and 24 grids, taking approximately two days to process (Figure 3). In the first round, optimal protein concentration, buffer, and pH conditions are determined. In the second round, detergents are further optimized. A condensed strategy is also provided using 8 grids and taking one day to process, which can be used when little sample is available or for use with side-entry or semiautomated sample loading systems like the Thermo Scientific™ Tundra™ Cryo-TEM.

The recommended screening strategies are designed for use with the Thermo Scientific™ Vitrobot™ Mark IV System and Thermo Scientific™ Krios™, Glacios™, and Talos Arctica™ microscopes with autoloader systems and EPU multigrid.

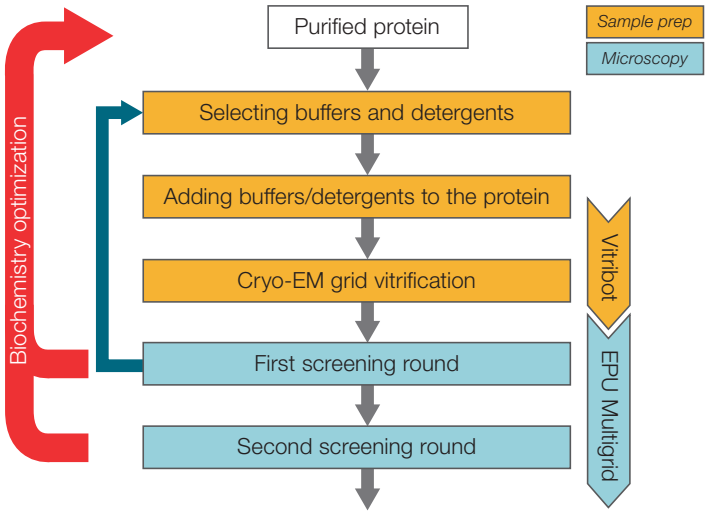


Figure 3. Comprehensive cryo-EM sample screening strategy.

VitroEase negative stains

Thermo Scientific™ VitroEase™ Methylamine Tungstate and Thermo Scientific™ VitroEase™ Methylamine Vanadate Negative Stains are easy, ready-to-use negative stains for electron microscopy (EM) analysis.

Highlights:

- Provided as a stable, ready-to-use 2% stain solution
- Easy to use with straightforward staining protocol
- Uniform, electron-dense stain for EM
- Dries without crystallization, ideal for EM grids

VitroEase Methylamine Tungstate Negative Stain is a ready-to-use stain with excellent spreading qualities with high density for good contrast. It wets both grid films and specimens well at neutral pH.

This stain does not damage delicate structures as much as phosphotungstate (PTA) and has been found to be useful for negative staining of macromolecules, viruses, and membranes.

VitroEase Methylamine Vanadate Negative Stain is a ready-to-use stain with excellent spreading properties at a near-neutral pH of 8. It produces a light, uniform negative stain for high-resolution fine structure visualization and does not denature protein samples. This stain is also stable and nonvolatile in the electron beam, making it more resistant than uranyl acetate negative stain.

Figure 4. TEM images of negative-stained apoferritin using the VitroEase Methylamine Tungstate and VitroEase Methylamine Vanadate Negative Stains.

Vitrobot Mark IV System

The Thermo Scientific™ Vitrobot™ Mark IV System assists in robustly preparing grids for Cryo-EM. The instrument is fast and easy to use and allows the preparation of cryo-EM grids for various cryo-EM applications and sample types, such as purified proteins for SPA, cells for cryo-ET, and crystals for MicroED.

Sample preparation is an important step in the cryo-EM workflow, and preparing good quality grids for subsequent imaging can be a daunting task. The Vitrobot Mark IV System helps the user by automating the vitrification process, while providing precise but flexible control over critical parameters. By making use of a temperature- and humidity-controlled climate chamber, the system provides a controlled environment for vitrification that prevents cooling and concentration artifacts that can be introduced with manual or "open space" vitrification systems.

Highlights:

- Fully automated, reproducible vitrification of aqueous suspensions
- Precise control of critical process parameters
- Enclosed environmental chamber
- High sample throughput
- Easy and flexible user interface
- Semiautomated grid transfer

Learn more at **thermofisher.com/em-sample-prep**

Test your protein research knowledge

Question: Which of the following are cryo-EM techniques? (Select all that apply.)

A. SILAC

B. SRM

C. SPA

D. SEM

Answer: C

Ordering information

Product	Quantity	Cat. No.
Sample prep reagents		
VitroEase Cryo-EM Training Kit	25 preparations	A51363
VitroEase Apoferritin Standard	5 x 20 mL	A51362
VitroEase Methylamine Tungstate Negative Stain	5 mL	A51036
VitroEase Methylamine Vanadate Negative Stain	5 mL	A51037
VitroEase Buffer Screening Kit	1 kit	A49856
Instrumentation		
Vitrobot Mark IV System	1 system	VITROBOT

For information on cryo-EM instruments, visit **thermofisher.com/emlifesciences**

Resources

You have come to expect high-quality products for your protein research from us, but our support does not stop there. We also provide technical tips, selection guides, and other helpful tools to make your protein research experiments go more smoothly.

Protein biology

Protein Analysis Learning Center

The Protein Analysis Learning Center is here to assist you in your quest to understand the proteome, whether you want to review the basics, gain more in-depth knowledge, or discover how to use the latest research tools. Find resources for the different protein analysis techniques starting from protein expression to quantitative mass spectrometry including webinars, articles, protocols, and troubleshooting tips.

Access the learning center at **thermofisher.com/proteinlearning**

Protein Degradation Resource Center

The Protein Degradation Resource Center includes the latest information and research tools to assist you in evaluating protein degradation by providing product offerings and solutions to your everyday protein degradation questions.

Access the resource center at **thermofisher.com/proteindegradation**

Antibodies Learning Center

Selecting individual antibody products from among hundreds of options can be a challenge. The Antibodies Learning Center contains educational material designed to empower researchers and technicians with the background knowledge about antibody technologies necessary to develop, select, and use antibodies to advance their research.

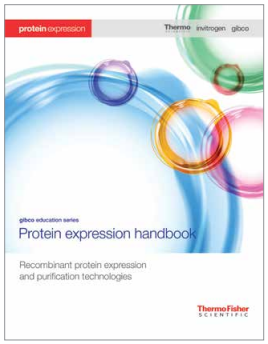
Access the learning center at **thermofisher.com/antibodies-learning-center**

Cloning and protein expression

Protein expression handbook

Download this handbook to help you choose the right protein expression system and purification technologies for your specific application and needs. Get tips and tricks when starting an experiment, and find answers to everyday problems related to protein expression.

Request your copy at **thermofisher.com/expressionguide**



Protein expression virtual short courses

Join our instructors who will discuss the concept, techniques, and optimization strategies for the rapid generation of milligram-to-gram quantities of secreted or intracellular recombinant proteins for therapeutic, functional, and structural studies. The set of courses combines instruction and case studies in an interactive environment.

Register at **thermofisher.com/gibcoproteinexpressionvirtualshortcourses**

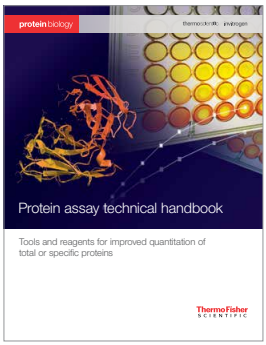
Protein Expression Basics online learning hub

Gibco™ Protein Expression Basics is an online hub that brings together educational content and tools to enhance your research. Here you can access our protein expression selection guide, handbook, educational videos, and technical resources. Start here if you are new to protein expression or need to refresh your knowledge.

Access the learning hub at **thermofisher.com/gibcoproteinexpressionbasics**

Protein sample preparation

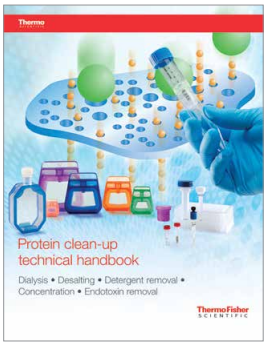
Protein assay technical handbook



Download this handbook to help select the appropriate protein assay method based on the assay time, sensitivity, compatibility, standard curve linearity, and protein-to-protein variation. Learn about our wide range of colorimetric (copper- or dye-based) and fluorescence protein assays, as well as our more specialized assays to quantify peptides, antibodies, protein modifications, or functional (enzymatic) classes of proteins. Discover tools and strategies to help optimize your protein quantitation assays to help ensure more accurate downstream results.

Download the free handbook at **thermofisher.com/protein-assay-handbook**

Protein clean-up technical handbook



Learn how to effectively remove contaminants, perform buffer exchange, or concentrate protein samples from 2 µL to 250 mL using various Thermo Scientific™ protein biology tools in this 48-page handbook. Dialyze protein samples securely using Slide-A-Lyzer cassettes and devices. Rapidly desalt samples with high protein recovery using Zeba desalting spin columns and plates. Efficiently extract specific contaminants using resins optimized for detergent or endotoxin removal. Concentrate dilute protein samples quickly using Pierce Protein Concentrators.

Download the free handbook at **thermofisher.com/proteincleanuphandbook**

Protein Sample Preparation eLearning Course

This free course comprises two interactive modules that were developed to provide a succinct, contextual overview of protein sample isolation using total protein extraction and subcellular fractionation. The module covers methods for purifying whole cell lysates and describes strategies for protein cleanup procedures.

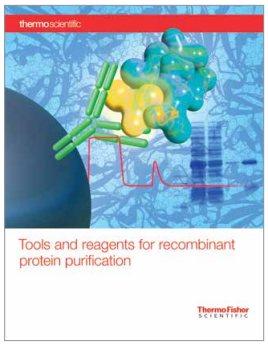
Access the free course at **thermofisher.com/elearningproteinsampleprep**

Protein purification

Tools and reagents for recombinant protein purification

Download this handbook to learn more about our wide portfolio of immobilized ligands for the purification of 6xHis-, GST-, c-Myc-, HA-, and DYKDDDDK (FLAG™)-tagged proteins. Our broad offering of magnetic beads, magnetic agarose, agarose, Superflow resins, and UltraLink™ resins enable purification and pull-down applications from low-microgram (high-throughput) to low-kilogram (pilot) scales. Our helpful selection guides and relevant data will help you select the best support to meet your needs.

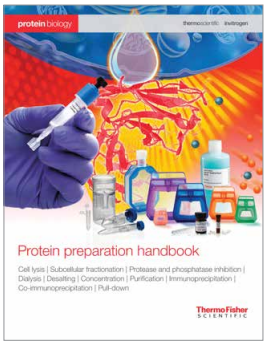
Download the free handbook at **thermofisher.com/tag-purification-handbook**



Protein preparation handbook

Discover tools for protein extraction, protein cleanup, protein purification, and immunoprecipitation that can help take your protein research to the next level.

Download the free handbook at **thermofisher.com/proteinprephandbook**

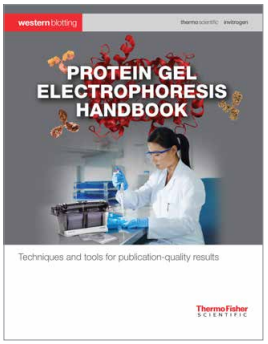


Protein gel electrophoresis

Protein gel electrophoresis handbook

This technical handbook lets you discover even more about protein gels, sample preparation, buffers and reagents, standards, electrophoresis chambers, power supplies, and staining.

Learn more at **thermofisher.com/pagehandbook**



Protein gel selection guide

Use our interactive product selector to find the right gel for your experiment.

Access the guide at **thermofisher.com/proteingelguide**

Protein gel conversion tool

Easily find Invitrogen™ gels to replace your current precast gels from another supplier.

Access the tool at **thermofisher.com/proteingelconversion**

Protein Standards and Ladders Selection Guide

Find the right protein ladder for your application and gel system. Use our interactive tool to see the migration of recommended protein ladders in different gel chemistries.

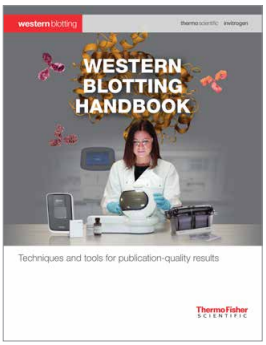
Learn more at **thermofisher.com/proteinladdersguide**

Western blotting

Western blotting handbook

This comprehensive, easy-to-use guide covers every procedural western blotting step, from gel transfer to hands-free blot processing systems, imaging and data analysis, multiplexing, western blot quantitation, and more.

Learn more at **thermofisher.com/westernhandbook**



Invitrogen™ BlotBuilder™ western blot product selection tool

Let us help you select the right tools specifically for your protein and experimental needs. Simply answer a few questions about your protein of interest and review a set of recommended products with a personalized western blot protocol.

Access the tool at **thermofisher.com/blotbuilder**

Protein Electrophoresis and Western Blotting Education Center

Gaining publication-quality results immediately is not exactly the norm when performing western blotting. Access resources to learn about protein gel electrophoresis and western blotting methods, from webinars to quick tips and tricks. Whether you are new to western blotting or an experienced researcher looking to expand your knowledge, consider this center to help you get better western results and succeed sooner.

Access resources at **thermofisher.com/westerneducation**

Immunoassays

ELISA troubleshooting guide

Is your ELISA not working? Invitrogen™ ELISA kits are built to be easy, but things can still go wrong. To help, we've put together a collection of troubleshooting resources with tips and tricks for the most common challenges.

Access the guide at **thermofisher.com/elisatroubleshooting**

ELISA protocols

General protocols are available for a standard sandwich ELISA using a 96-well plate for colorimetric (chromogenic) or chemiluminescent detection.

View the protocols at **thermofisher.com/generalelisaprotocol**

ELISA selection guide

With thousands of ELISA kits to choose from, we're confident you'll find the right kit for you.

Find your ELISA at **thermofisher.com/elisa**

ELISA validation and quality control

Validation and quality control are critical aspects for creating reliable ELISA kits. That's why we put our kits through rigorous testing to ensure they work for you time and time again.

Learn more about ELISA validation and QC testing at **thermofisher.com/elisavalidation**

Biomarker quantitation assay guide

This guide describes all the options for single- and multiple-biomarker quantitation assays for both protein and mRNA.

Learn more at **thermofisher.com/biomarkerguide**

Luminex how-to videos

Watch how-to videos to learn tips and techniques for running Luminex® assays.

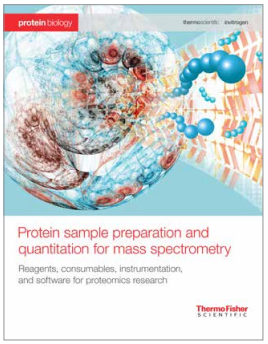
Learn more at **thermofisher.com/immunoassayvideos**

Mass spectrometry

Protein sample preparation and quantitation for mass spectrometry

This 168-page handbook provides an integrated overview of protein sample preparation, quantitation, and instrument calibration for optimized proteomics workflows using mass spectrometry. Guidelines for selecting the right chromatography products, mass spectrometers, and software for discovery and targeted proteomics applications are provided.

Download at **thermofisher.com/mshandbook**



Mass spec analysis technical resources

Find a collection of valuable tools to help improve your mass spectrometry results, including handbooks, application-specific white papers, technical notes, late-breaking posters, and helpful webinars.

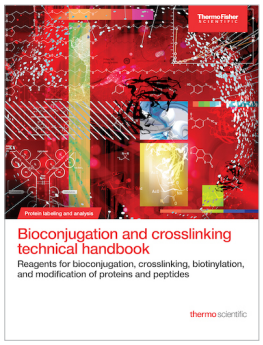
Access now at **thermofisher.com/msresources**

Protein modification and crosslinking

Bioconjugation and crosslinking technical handbook

This handbook discusses reagents for bioconjugation, crosslinking, biotinylation, and modification of proteins and peptides.

Download at **thermofisher.com/bioconjugationhandbook**



Biotinylation Reagent Selection Tool

This tool provides quick access to customized lists of Thermo Scientific™ biotinylation and desthiobiotin reagents that meet specific criteria, including target functional group, spacer arm length, and solubility. Use the simple dropdown boxes to easily select the optimal biotinylation reagent for your application.

Learn more at **thermofisher.com/biotinylation-tool**

Crosslinker Selection Tool

This tool provides quick access to customized lists of Thermo Scientific™ Pierce™ crosslinkers that meet specific criteria, including target functional group, solubility, and the ability to permeate the cell membrane. Use the simple dropdown boxes to easily select the optimal crosslinking reagent for your application.

Learn more at **thermofisher.com/crosslinking-tool**

Antibody Labeling Kits Selection Guide

This guide provides quick access to customized lists of antibody labeling kits that meet specific criteria, including type of label, chemical reactivity, and labeling scale. Use this interactive selection guide to find the right antibody or protein labeling kit for your experiment.

Learn more at **thermofisher.com/ABlabelingguide**

Cryo-EM sample preparation

eBook: Getting started with cryo-EM

Learn more about adopting cryo-EM and how it can advance your research in our new eBook. Created especially for structural biologists and biochemists who do biomedical research and are interested in obtaining deeper structural and functional insights of macromolecules.

Download the eBook at **thermofisher.com/cryo-em-ebook**

eBook: Structure and function with cryo-tomography

Cryo-electron tomography allows researchers to access the inner-workings of cells in 3D and at unprecedented nanoscale resolution. Download our eBook to explore a curated collection of scientific publications highlighting the use of cryo-electron tomography for applications in cell biology, neuroscience, microbiology, and virology.

Download the book at **thermofisher.com/tomographyebook**

Cryo-EM Learning Center

The Cryo-EM Learning Center provides the latest information on cryo-electron microscopy (cryo-EM). This resource hub contains rich and reliable technical content designed for new and experienced researchers who are exploring the capabilities of cryo-EM.

Access the learning center at **thermofisher.com/cryo-em**

Learn more at **thermofisher.com/proteinbiology**