protein biology

thermo scientific invitrogen

Protein preparation handbook

Cell lysis • Subcellular fractionation • Protease and phosphatase inhibitors • Dialysis • Desalting • Concentration • Purification • Immunoprecipitation

ThermoFisher
SCIENTIFIC

Extract. Clean up. Purify. Immunoprecipitate.

We offer a full range of optimized reagents for efficient protein extraction and fractionation as well as the targeted inhibition of unwanted protease and phosphatase activity. Our convenient devices and high-performance affinity resins and magnetic beads enable maximum yield for the purification, enrichment, and clean-up of proteins and antibodies for downstream applications.

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. Other important considerations include the preservation of protein activity and function as well as the reduction of background effects.

- **Tissue and cell lysis.** Historically, mechanical disruption has been used to lyse cells and tissues; our gentle, detergent-based solutions have been developed to efficiently lyse cells and enable the separation of subcellular structures without requiring physical disruption, providing high yield of active proteins.
- **Detergent solutions.** Detergents are frequently used in cell lysis reagent formulation and other protein research methods. Thermo Scientific™ Surfact-Amps™ Detergent Solutions are highly purified, precisely diluted (10%) formulations that are ideal for applications or assays that are sensitive to contaminants that are present in unpurified detergents.
- **Protein stabilization.** 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. Thermo Scientific™ Halt™ and Thermo Scientific™ Pierce™ Protease and Phosphatase Inhibitor Cocktails and Tablets are broad-spectrum blends in both liquid (100X) and tablet formats for complete protein protection during extraction.

Protein clean-up

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 or subsequent sample processing, such as protein purification.

- **Dialysis.** Dialysis is a classic separation technique that facilitates the removal of small, unwanted compounds from proteins in solution by selective diffusion through a semipermeable membrane. Proteins that are larger than the membrane pores are retained on the sample side of the membrane, but low molecular weight contaminants diffuse freely through the membrane and can be removed over multiple buffer exchanges. Traditionally, flat dialysis tubing has been utilized, which requires preparation, and is slippery and cumbersome to handle. Thermo Scientific™ Slide-A-Lyzer™ dialysis cassettes and devices are ready to use and designed to eliminate potential sample leakage and maximize ease of use for specific applications.

- **Desalting.** Size-exclusion chromatography (also known as gel filtration) can be effectively utilized for protein desalting. A resin is selected 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 small contaminants to slow down their rate of migration as they get trapped in the resin, while the larger, faster proteins emerge from the column first, allowing the protein of interest to be recovered separately from the small molecules retained on the column. Thermo Scientific™ Zeba™ desalting products contain a unique resin and were specifically designed to provide consistent performance over a wide range of protein concentrations and sample sizes. High protein recovery can be achieved even for dilute protein samples.
- **Concentration.** Protein concentration and diafiltration, similar to dialysis, uses a semipermeable membrane to separate macromolecules from low molecular weight compounds. Unlike dialysis, which relies on passive diffusion, concentration is achieved by forcing both liquid (buffers) and low molecular weight solutes through the membrane by centrifugation where they are collected on the other side (filtrate). Macromolecules remain on the sample side of the membrane, where they become concentrated to a smaller volume (retentate). For buffer exchange, the retentate is diluted to the original volume with exchange buffer and centrifuged multiple times until the desired level of exchange has been achieved. Our high-performance Thermo Scientific™ Pierce™ Protein Concentrators enable rapid sample processing with high protein recovery.

Protein purifi ation

Various methods are used to enrich or purify a protein of interest from other proteins and components in a crude cell lysate or other sample. Ion exchange and affinity chromatography are two commonly used strategies for partial or 1-step purification.

Ion exchange (IEX) chromatography. This purification method enables the separation of proteins based on the protein charge at a particular pH. Since multiple proteins may have similar charges, ion exchange chromatography generally enables only partial purification of a protein of interest when used early in a multistep purification process; however, IEX resins can also be used during a final polishing step to remove specific contaminants that persist after other purification steps. Typically, proteins bind to the column at low ionic strength and elute differentially by increasing salt concentration or changing pH in a gradient. A cation exchange resin binds to positively charged proteins; an anion exchange resin binds to negatively charged proteins. Ion exchange resins are classified as “weak” or “strong”, which refers to the extent that the ionization state of the functional groups varies with pH.

Affinity chromatography. This purification method is enabled by the specific binding properties 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 is desirable because it often produces higher protein yields and requires less steps than other purification methods. It is the method of choice for purifying recombinant or biotinylated proteins and antibodies.

Our high-performance resins are available with a range of ligand chemistries and in formats for purifying from microgram to kilogram quantities of protein.

Immunoprecipitation

Immunoprecipitation (IP) is the small-scale affinity purification of antigens using a specific antibody that is immobilized to a solid support such as magnetic beads or agarose resin. IP 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 and other assay techniques. Other similar techniques used to study protein interactions include co-immunoprecipitation (co-IP), which is similar to IP except that the target antigen precipitated by the antibody is used to co-precipitate its binding partner(s) or associated protein complex from the lysate, and pull-downs, which are used when antibodies to specific proteins are not available. These “bait” proteins are tagged with an epitope to which a high-affinity antibody is available and ectopically expressed in the cell of interest.

Our magnetic beads provide fast and reproducible sample processing with high protein yields and low nonspecific binding using antibody, biotin, or recombinant tag ligands, as well as activated surface beads for custom immobilization.

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. These gentle formulations have been validated 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, EMSA, and enzyme assays.

Highlights:

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

Table 1. Overview of sample types and Thermo Scientific™ 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 • Lysosome Enrichment Kit	• Cell Surface Protein Isolation Kits • Syn-PER Reagent
	**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, roots, flowers)**	Total protein extraction	• P-PER Reagent	

Comparison of cross-contamination between subcellular fractions

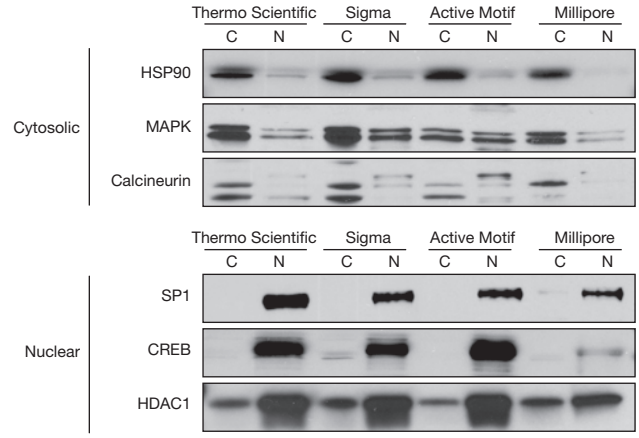


Figure 1. Nuclear and cytosolic fractions are obtained with minimal cross-contamination. HeLa cells were extracted with the Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Reagents or with nuclear extraction kits from other vendors. Samples of the nuclear and cytosolic fractions were analyzed by western blot using antibodies against common nuclear, cytoplasmic, and membrane protein markers and visualized using Thermo Scientific™ SuperSignal™ West Pico Chemiluminescent Substrate (Cat. No. 34080). Nuclear fractions produced with the NE-PER kit had minimal to no contamination with cytosolic or membrane proteins.

Comparison of protein yield

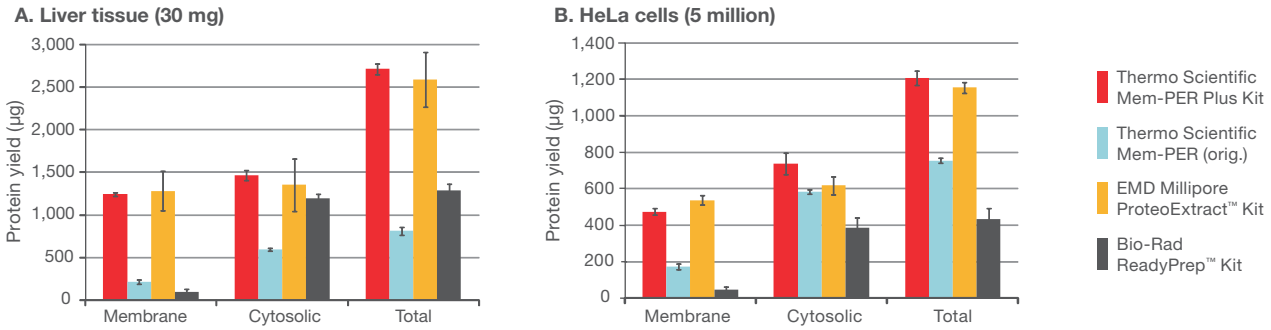


Figure 2. Improved protein yield using the Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit (Cat. No. 89842). Membrane proteins were isolated from mouse liver tissue and HeLa cells using four commercial extraction kits. Protein yields (micrograms) for membrane, cytosolic, and total fractions were determined with the Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Cat. No. 23225).

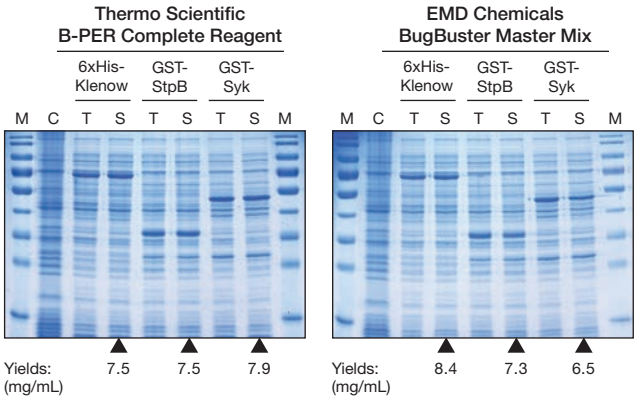


Figure 3. Protein yield comparison of two bacterial cell lysis reagents. *E. coli* ER2566/pLATE51-Klenow, ER2566/pGST-CC-StpB, and ER2566/pGS-Syk cell pellets (0.5 g), were resuspended in 2.5 mL aliquots of Thermo Scientific™ B-PER™ Complete Bacterial Protein Extraction Reagent (Cat. No. 89821) or BugBuster™ Master Mix with gentle vortexing for 15 minutes at room temperature. Insoluble cell debris was removed by centrifugation at 16,000 x *g* for 20 minutes at 4°C. Protein yields (concentrations) for soluble fractions were determined using the Pierce BCA Protein Assay Kit (Cat. No. 23225).

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. These formulations (10% w/v) provide high purity, quality, and stability. Unlike neat detergents, which are extremely viscous, Surfact-Amps 10% solutions are easy to pipette and accurately dispense. The surfactant solutions are carefully prepared and packaged under nitrogen in glass ampules or nonleaching 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 1.0 µeq/mL peroxides and carbonyls
- **Stable**—packaged under inert nitrogen gas in glass ampules or HDPE bottles

Table 2. Properties of common detergents.

Detergent	Description	Aggregation number	Micelle MW	MW	Critical micelle concentration (CMC, mM)	CMC w/v (%)	Cloud point (°C)	Dialyzable
Triton X-100	Nonionic	140	90,000	647	0.24	0.0155	64	No
Triton X-114	Nonionic	—	—	537	0.21	0.0113	23	No
NP-40	Nonionic	149	90,000	617	0.29	0.0179	80	No
Brij-35	Nonionic	40	49,000	1,225	0.09	0.1103	>100	No
Brij-58	Nonionic	70	82,000	1,120	0.077	0.0086	>100	No
Tween-20	Nonionic	—	—	1,228	0.06	0.0074	95	No
Tween-80	Nonionic	60	76,000	1,310	0.012	0.0016	—	No
Octyl glucoside	Nonionic	27	8,000	292	23–25	0.6716–0.7300	>100	Yes
Octylthio glucoside	Nonionic	—	—	308	9	0.2772	>100	Yes
SDS	Anionic	62	18,000	288	6–8	0.1728–2304	>100	Yes
CHAPS	Zwitterionic	10	6,149	615	8–10	0.4920–0.6150	>100	Yes

Table 3. Purity comparison of Tween-20 detergents.*

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

Table 4. Purity comparison of NP-40 detergents.*

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

* Oxidant levels were measured using Thermo Scientific™ Pierce™ Quantitative Peroxide Kit (Cat. No. 23385) and carbonyl levels were measured using the Brady test for carbonyls.

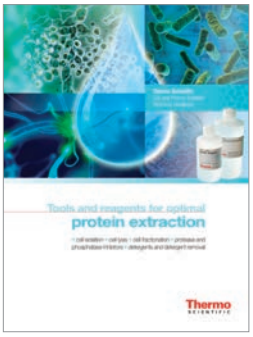
Table 5. Purity comparison of Triton X-100 detergents.*

Manufacturer/ brand	Peroxide concentration (µeq/mL)	Carbonyl concentration (µeq/mL)
Thermo Scientifi	≤0.20	≤0.20
Amresco	≤0.20	≤0.20
Anatrace	≤0.20	0.333
G-Bioscience	≤0.20	≤0.20
Millipore EMD	≤0.20	≤0.20
Roche	≤0.20	0.253
Sigma	≤0.20	0.355

Table 6. Purity comparison of Brij-35 detergents.*

Manufacturer/ brand	Peroxide concentration (µeq/mL)	Carbonyl concentration (µeq/mL)
Thermo Scientifi	<0.035	<0.62
Amresco	1.075	3.742
Anatrace	<0.035	<0.62
G-Bioscience	<0.035	<0.62
Millipore EMD	<0.035	<0.62

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



Download our Cell and Protein Isolation Technical Handbook. Learn how to optimize protein extraction from cells and tissues for better yield and improved downstream compatibility using our protein extraction and subcellular fractionation reagents and protease and phosphatase inhibitor cocktails and tablets. Improve your protein biology methods with our highly purifi d and precisely diluted detergent solutions.

thermofisher.com/proteinextractionhandbook

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 divalent cation–sensitive assays.

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 of one-year shelf life
- **Complete protection**—combined cocktail available with all-in-one formulations containing both protease and phosphatase inhibitors
- **Compatible**—use directly with Thermo Scientific™ Pierce™ Cell Lysis Buffers or other commercial or homemade detergent-based lysis reagents

Table 7. 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-glycero-phosphate	Serine/threonine phosphatase		●	●
Sodium pyrophosphate	Serine/threonine phosphatase		●	●

* EDTA not in EDTA-free formulations.

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

Comparison of protease or phosphatase inhibition

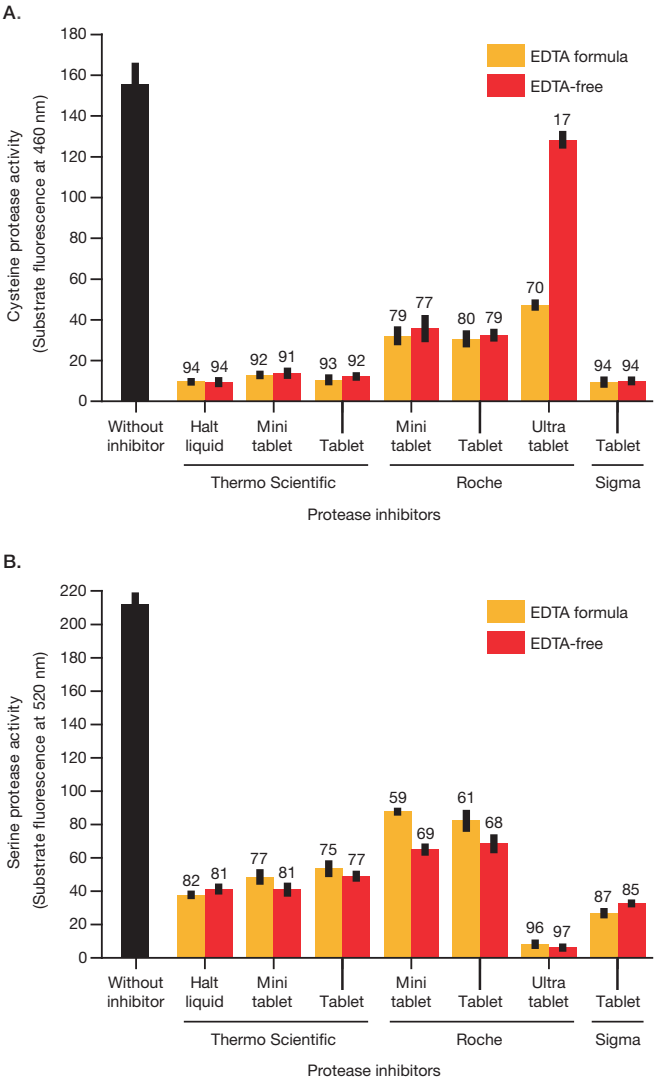


Figure 4. Comparison of commercially available protease inhibitor cocktails and tablets. Pancreatic extract (50 µL; 1 µg/µL protein) or trypsin (25 µL, 0.1 units/µL) was incubated with a quenched-fluorescent, protease-cleavable substrate for cysteine **(A)** or serine proteases **(B)** in the presence or absence of commercially available protease inhibitors with EDTA-containing (orange) or EDTA-free (red) formulations. Reactions were incubated for two hours at 37°C and the fluorescence determined at the indicated detecting emissions. The percent protease inhibition is shown for each protease inhibitor formulation.

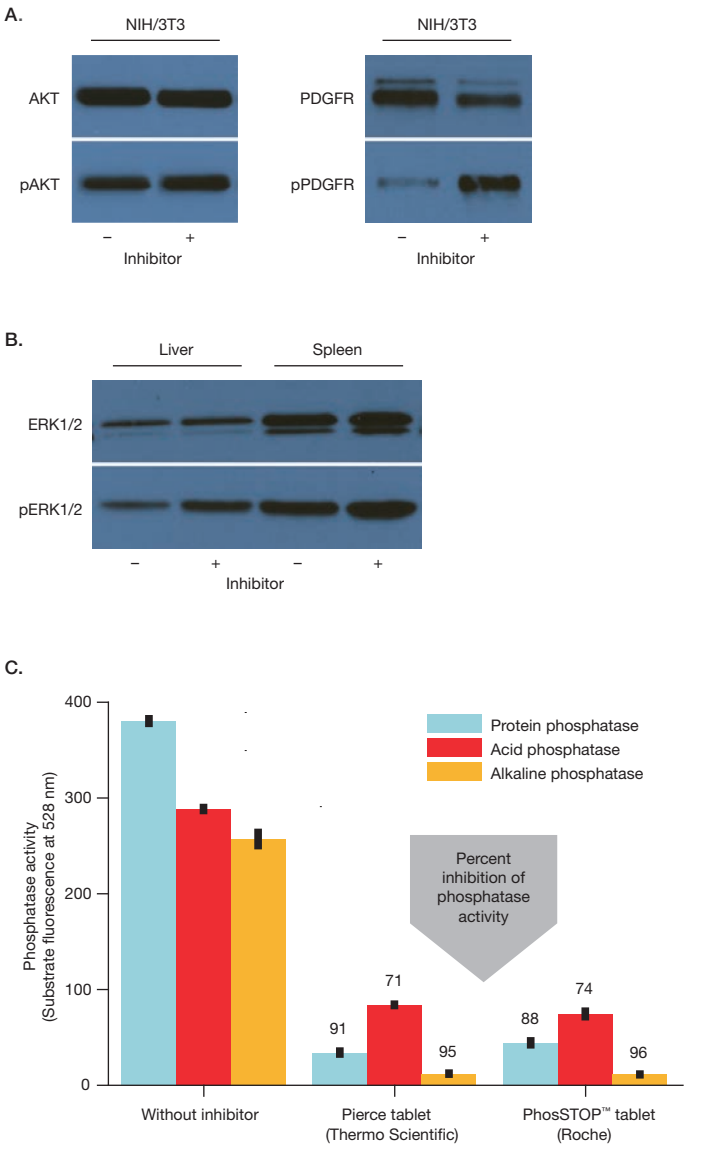
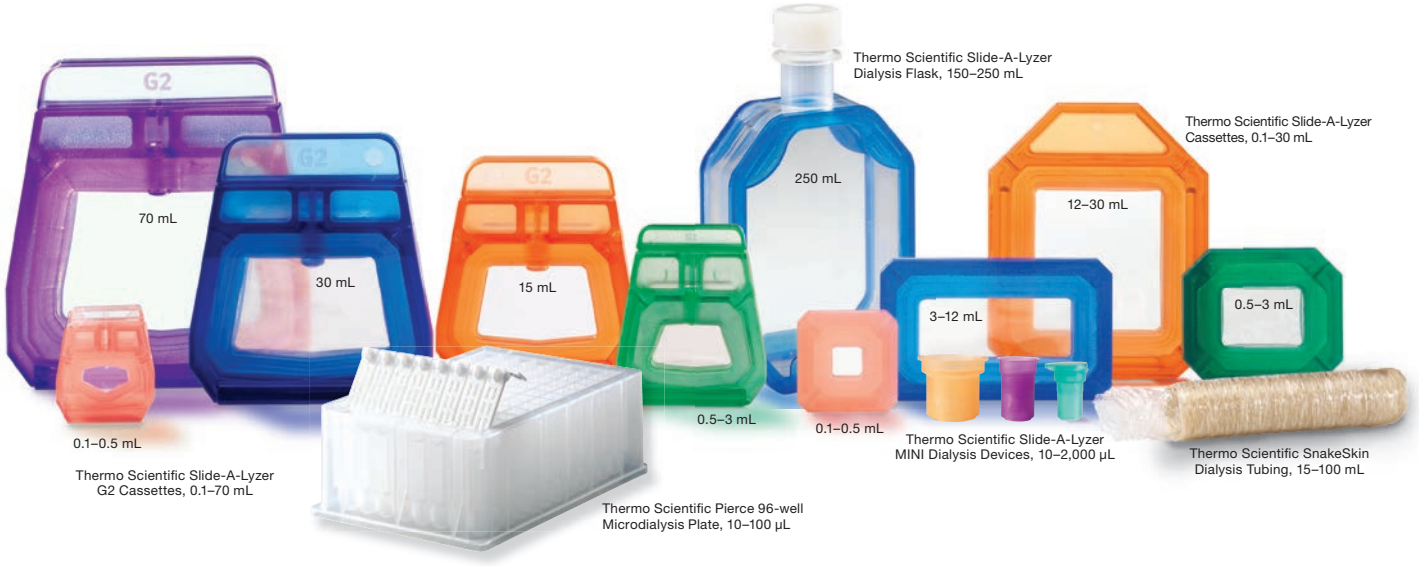


Figure 5. Protein phosphorylation is preserved in cell and tissue extracts. Relative levels of total and phosphorylated protein from extracts prepared in the absence or presence of phosphatase inhibitors were determined by western blot analysis. **(A):** AKT and PDGFR in serum-starved, PDGF-stimulated (100 ng/mL) NIH/3T3 cell extracts. **(B):** ERK1/2 in liver and spleen tissue extracts. **(C):** the degree of inhibition for protein, acid, and alkaline phosphatase activity was determined in mouse brain extract after treatment with Pierce Phosphatase Inhibitor Tablets or another commercially available phosphatase inhibitor tablet. Percent inhibition is indicated.

Slide-A-Lyzer dialysis products

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



Thermo Scientific™ dialysis units help facilitate the 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™ Dialysis Cassettes (original and G2) are recommended for small to medium volumes, and Slide-A-Lyzer™ Dialysis Flasks are recommended for larger volumes.

Highlights:

- **Excellent sample recoveries**—low-binding plastic and membranes help minimize sample loss compared to filtration and resin systems
- **Convenient**—easy-to-grip format helps simplify sample addition and removal with syringe and/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 8. Thermo Scientific™ high-performance dialysis product selection guide.

MWCO membrane	10–100 µL Pierce 96-well Microdialysis Plate	10–2,000 µL Slide-A-Lyzer MINI Dialysis Device	0.1–70 mL Slide-A-Lyzer G2 Dialysis Cassette	0.1–30 mL Slide-A-Lyzer Dialysis Cassette	150–250 mL Slide-A-Lyzer Dialysis Flask	15–100 mL SnakeSkin Dialysis Tubing
						
2K	NA	✓	✓	✓	✓	NA
3.5K	✓	✓	✓	✓	✓	✓
7K	NA	✓	✓	✓	NA	✓
10K	✓	✓	✓	✓	✓	✓
20K	NA	X	X	X	X	NA

Protein recovery by molecular weight cutoff (MWCO)

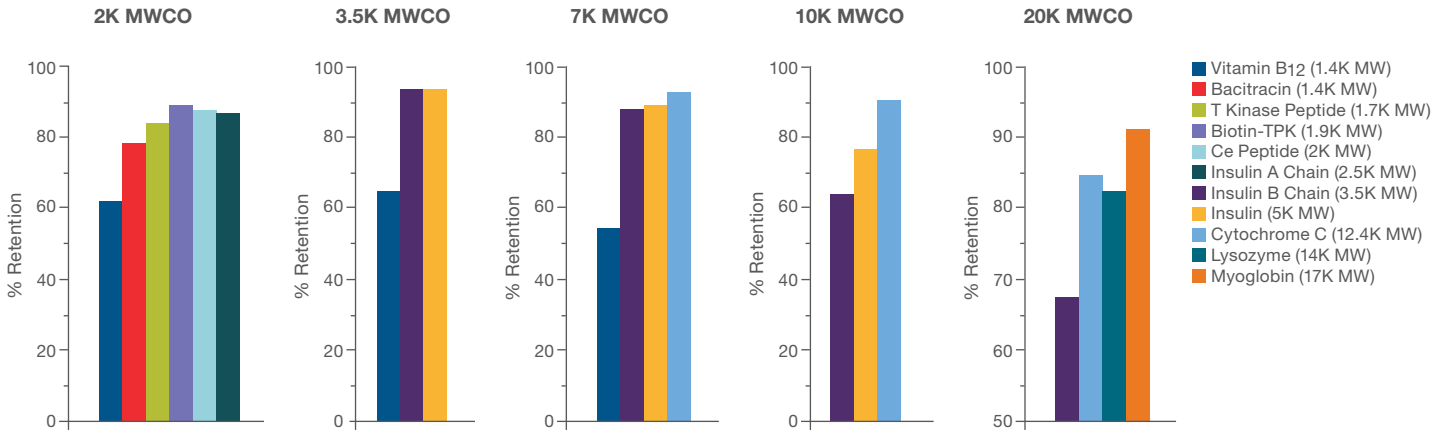


Figure 6. Sample retention by the 2K, 3.5K, 7K, 10K, and 20K MWCO Thermo Scientific™ Slide-A-Lyzer™ Cassette membrane. Individual proteins or vitamin B_{12} (1 mg/mL) in either saline or 0.2 M carbonate bicarbonate buffer, pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of retentate was estimated using either the Pierce BCA Protein Assay Kit or absorption at 360 nm (for vitamin B_{12}).

Dialysis rates for various formats

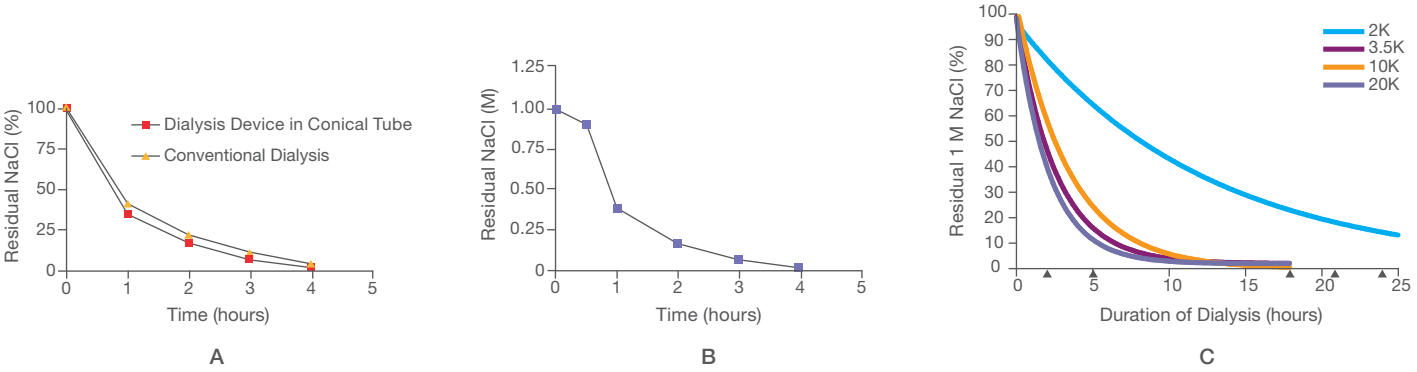


Figure 7. The rate of removal of NaCl using various dialysis products. NaCl removal from samples was determined by measuring the conductivity of the retentate at the indicated times. **Panel A:** Slide-A-Lyzer MINI Dialysis Device (10K MWCO, 2 mL) versus conventional dialysis. Bovine serum albumin (BSA) 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 hours. Results are the average of two samples. For conventional dialysis, the samples were dialyzed against 2 L of water in a beaker with stirring. Greater than 95% of NaCl was removed within 4 hours.

Panel B: Samples of 0.1 mL (0.4 mg/mL cytochrome C containing 1 M NaCl) were dialyzed in the Pierce 96-well Microdialysis Plate against 1.8 mL of water at RT with gentle shaking. The buffer was changed at 1-, 2-, and 3-hour intervals over a 4-hour period. Removal of NaCl was >83% after 2 hours and >99% after 4 hours. **Panel 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, and 20K MWCOs. The dialysis buffer (4 L) was changed after 2 and 5 hours (triangles; also at 41 hours for the 2K condition). Greater than 95% of NaCl was removed within 8 to 18 hours (41 hours for the 2K condition).

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

Zeba desalting products

Convenient spin column and plate 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.

Highlights:

- **High performance**—proprietary resin enables excellent protein recovery and efficient contaminant removal
- **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

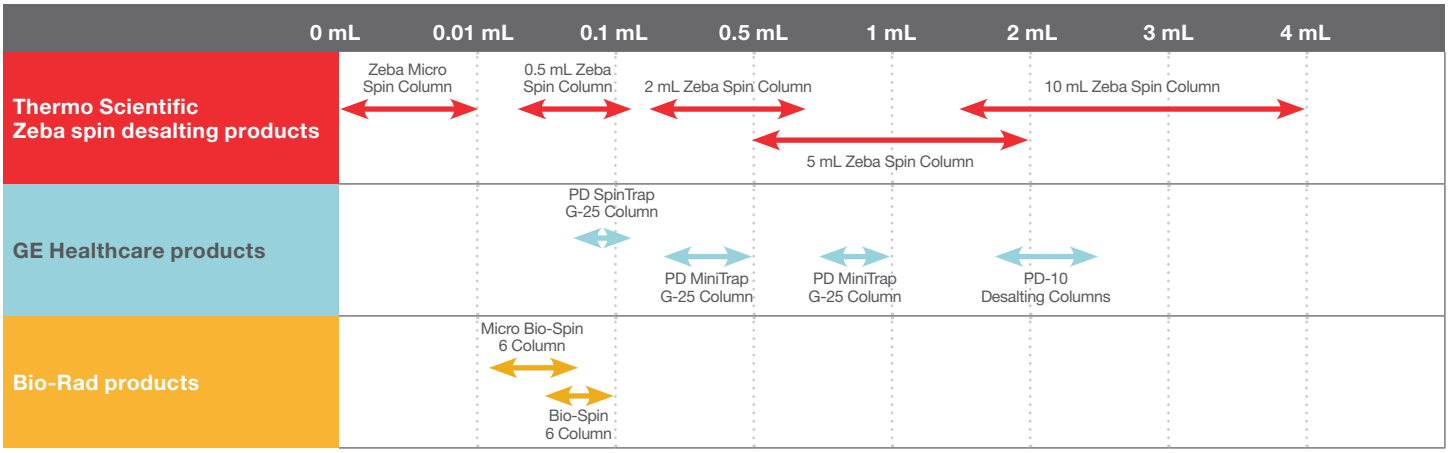
Table 9. Zeba desalting products selection guide by format and recommended sample volume.

Format	Micro spin column	0.5 mL spin column	2 mL spin column	5 mL spin column	10 mL spin column	96-well spin plate	1 mL chroma-tography column	5 mL chroma-tography column
								
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 10. 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		+

Table 11. Comparison of recommended sample volume capacity of common spin desalting products.



Comparison of protein recovery and sample dilution

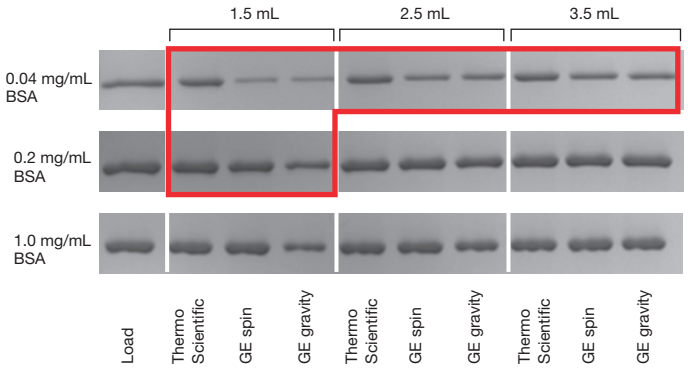


Figure 8. Zeba Spin Desalting Columns provide a high protein recovery while providing minimal sample dilution over a wider range of sample concentrations and volumes compared to alternative products. Zeba Spin Desalting Columns, 10 mL (7K MWCO) (Cat. No. 89893) and GE PD-10 Columns were used to desalt 1.5, 2.5, and 3.5 mL BSA samples at a concentration 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 GE PD-10. 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 highlighted 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™ concentrators are easy-to-use centrifugal devices that provide fast processing and excellent recovery of protein samples. These disposable ultrafiltration devices contain a polyethersulfone (PES) membrane in five distinct molecular weight cutoffs (MWCOs) for the concentration, desalting, and buffer exchange of biological samples, such as tissue culture media, antisera, monoclonal antibody preparations, and chromatography fractions. They can also be used to remove unincorporated label following protein modification or crosslinking reactions.

Highlights:

- **Rapid processing**—unique design minimizes membrane fouling, and sample concentration of 10- to 30-fold can be achieved in 5–30 minutes for 10K MWCO (device-dependent—times may vary for other MWCOs), even with particle-laden solutions
- **High recovery**—retain >90% of protein samples while removing contaminants or exchanging buffers
- **Convenient**—clear markings, wide sample chamber, and removable filtrate chamber make handling simple and easy
- **Instrument compatible**—can be used with standard centrifuges utilizing either fixed-angle or swinging-bucket rotors

Table 12. Pierce Protein Concentrators selection guide.

Volume range	0.1–0.5 mL	2–6 mL	5–20 mL	20–100 mL
				
MWCOs available	3K, 10K, 30K, 100K	3K, 10K, 30K, 100K	3K, 10K, 30K, 100K	5K, 10K, 30K, 100K
Processing time*	3–15 min	15–90 min	15–60 min	15–90 min
Retentate volume range*	9–67 µL	51–174 µL	121–777 µL	1.9–3.5 mL
Protein recovery range*	95–100%	94–100%	94–100%	92–98%

* Four different protein solutions were used for each molecular weight cutoff (MWCO).

Protein recovery compared to other suppliers

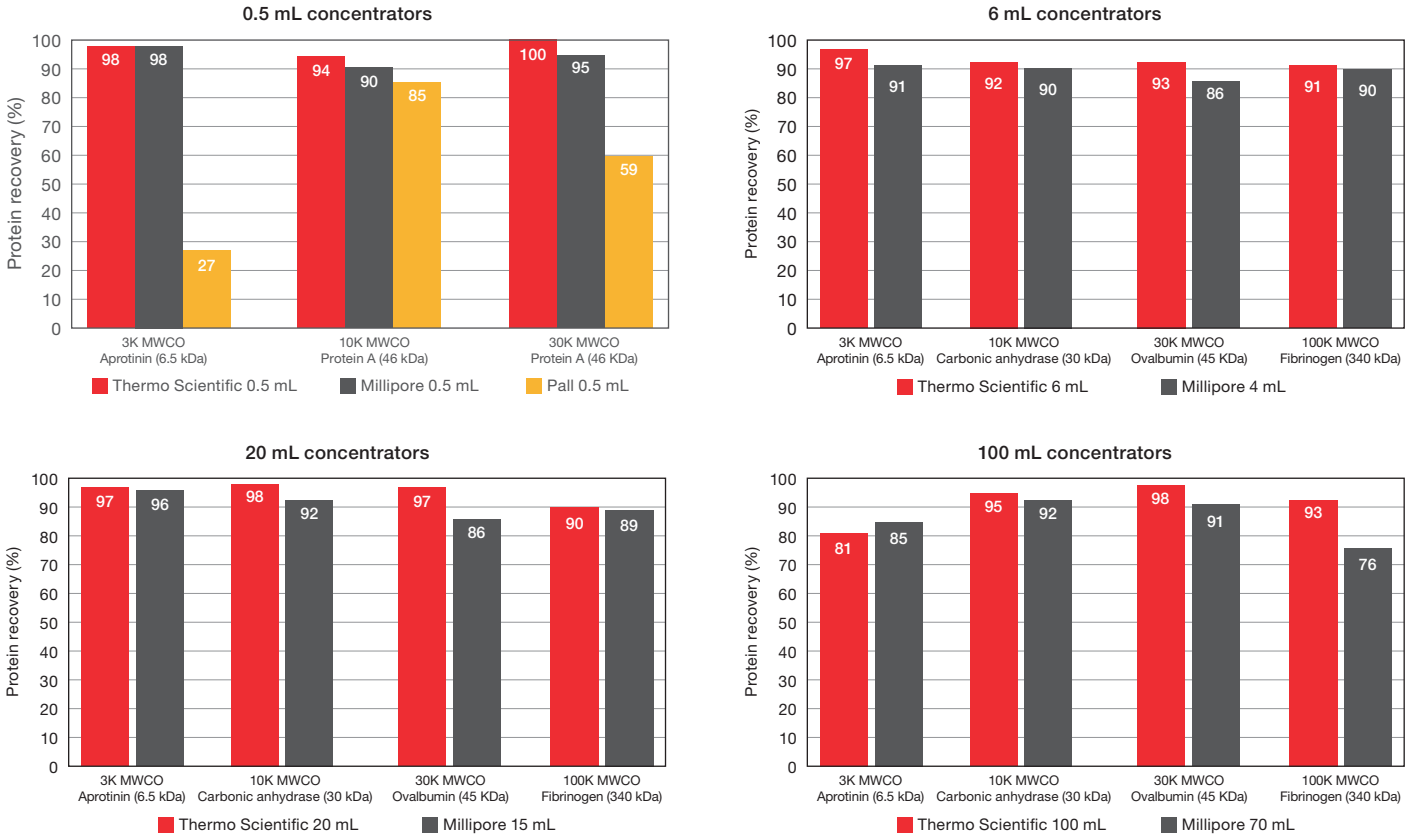
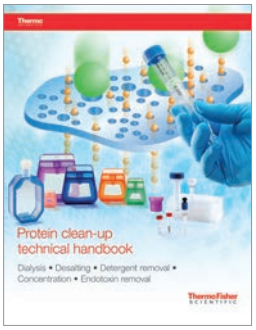


Figure 9. Comparison of protein recovery between Pierce Concentrators (using 3K, 5K, 10K, 30K, or 100K MWCO) and other vendors for 0.5 mL, 6 mL, 20 mL, and 100 mL concentrators. Samples of different protein solutions were centrifuged in Pierce Protein Concentrators and other suppliers' concentrators according to manufacturer instructions: 0.5 mL (15,000 x *g*), 6 mL (4,000 x *g*), 20 mL (4,700 x *g*), and 100 mL (1,200 x *g*). Samples were centrifuged until a greater than 15 to 30-fold decrease in sample volume was achieved; protein concentration was measured by either Pierce BCA Protein Assay Kit (0.5 mL concentrators only) or absorbance at A_{280}.

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



Learn how to effectively remove contaminants, buffer exchange, or concentrate protein samples from 2 µL–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.

thermofisher.com/proteincleanuphandbook

Protein purification products

High-performance resins and magnetic beads for maximum protein yield

The Thermo Scientific™ protein purification portfolio offers a broad range of products for the ion exchange and affinity-based isolation of proteins and antibodies from µg to kg quantities. Strong anion or cation exchange resins provide an intermediate level of purification during multistep isolation or act as a polishing step during the final stages of purification. Biotinylated or recombinant proteins can be conveniently captured using avidin or affinity tag–based binding supports. Customized protein purification can be achieved by immobilizing ligands to the appropriate activated support. Accessory products are available for increased convenience, including disposable columns and binding and elution buffers. Rapid screening or immunoprecipitation (IP), co-IP, and pull-down applications can be completed utilizing magnetic bead–based resins and kits, as described on pages 20–23.

Highlights:

- **Broad product selection**—strong ion exchange and affinity supports for the purification and enrichment of proteins and antibodies; affinity ligands enable 1-step purification of recombinant and biotinylated proteins, while activated supports provide a platform for custom protein immobilization

- **High performance**—resins are designed to maximize protein yield and reduce background
- **More formats**—magnetic beads, loose resin, FPLC cartridges, and 96-well filter plates enable protein purification from screening and small-scale phases to process-scale purification
- **Economical**—pricing that is similar to or better than leading competitors

Table 13. Overview of ion exchange, affinity, and activated supports.

Application	Purity level	Ligand/chemistry	Base bead type	Packaging options
Ion exchange purification	Medium to high (application specific)	Strong anion exchange Strong cation exchange	POROS	Loose resin
Antibody purification	High	Protein A, protein G, protein A/G	Agarose, magnetic beads, 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, cobalt, glutathione	Agarose, Superflow, magnetic beads	Loose resins or beads, spin columns and kits, chromatography cartridges, 96-well spin plates
		Anti–c-Myc, anti-HA	Agarose, magnetic beads	Loose resin 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 resin or dry powder
		Epoxy, tosylactivated, carboxylic acid, amine	Magnetic bead	Loose beads

Table 14. Select your resin based on purification scale and application.

Scale	Screening	Batch	Pilot	Process
Description	• Small scale • Automation-compatible	• Lab or bench scale	• Scale-up desired	• Production scale
Yield	• Microgram	• Milligram	• Gram	• Kilogram
Formats	• Magnetic particle processor • 96-well spin plate (agarose)	• Gravity flo • Spin columns (agarose, Superflow	• FPLC at medium flow rate	• FPLC at high flow rate
Application	• High-throughput screening • Interaction studies • Mutational analysis	• Functional assays • Structural analysis	• Structural analysis	• Bulk production
Recommended resin type	Magnetic beads Agarose	Agarose Superflo POROS	Superflo POROS	Superflo POROS

Ion exchange chromatography resins

We offer strong cation exchange (SCX) and strong anion exchange (SAX) resins, composed of a rigid polymeric bead with covalent surface chemistries, for easier handling and packing, and superior physical and chemical stability, resulting in a robust manufacturing process.

Strong cation exchange (SCX) resin

Thermo Scientific™ POROS™ XS Resin is the first high-capacity, high-resolution strong cation exchange resin that allows loading to more than 100 mg/mL capacity in the presence of up to 150 mM NaCl, while delivering unprecedented separation capability.

Comparison of resolution at different flow rates

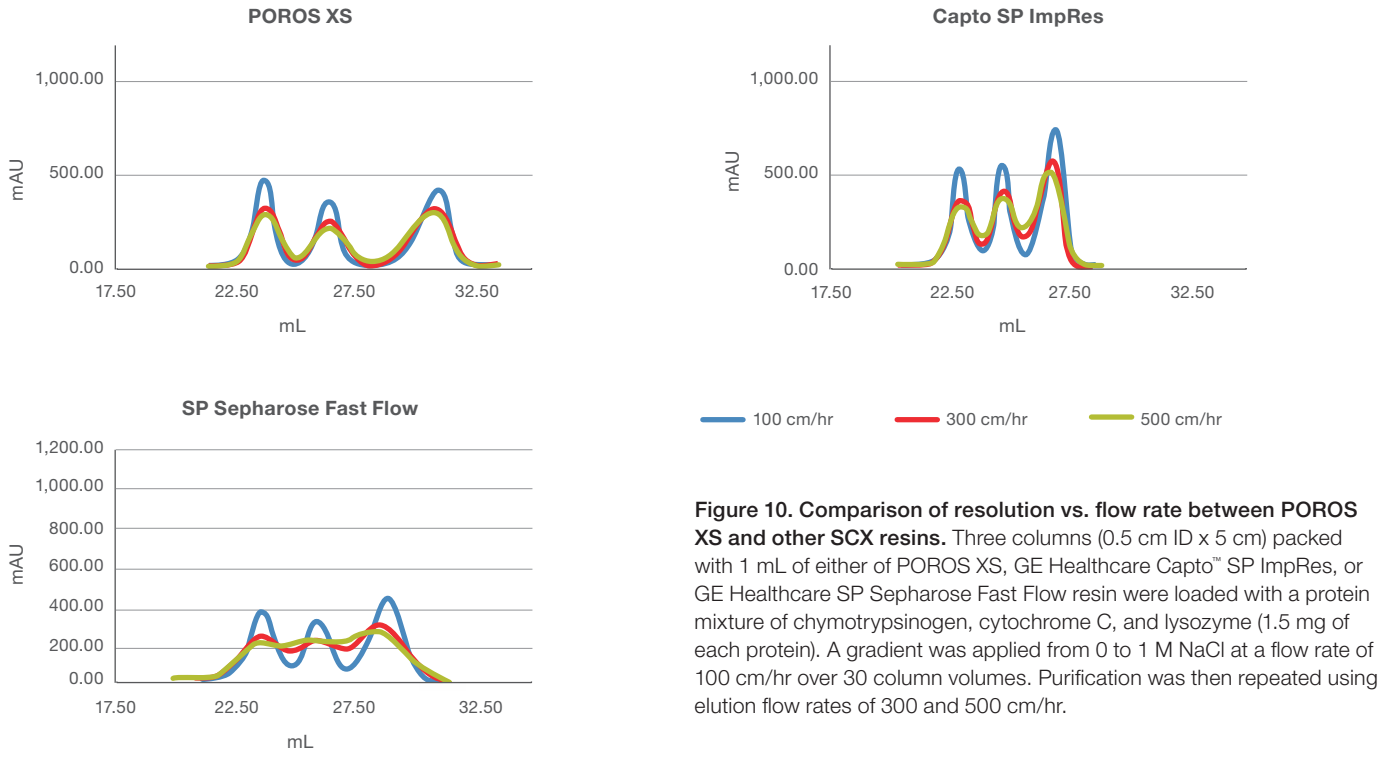


Figure 10. Comparison of resolution vs. flow rate between POROS XS and other SCX resins. Three columns (0.5 cm ID x 5 cm) packed with 1 mL of either of POROS XS, GE Healthcare Capto™ SP ImpRes, or GE Healthcare SP Sepharose Fast Flow resin were loaded with a protein mixture of chymotrypsinogen, cytochrome C, and lysozyme (1.5 mg of each protein). A gradient was applied from 0 to 1 M NaCl at a flow rate of 100 cm/hr over 30 column volumes. Purification was then repeated using elution flow rates of 300 and 500 cm/hr.

Strong anion exchange (SAX) resins

The Thermo Scientific™ POROS™ XQ resin is a next-generation, high-capacity, high-resolution, salt-tolerant strong anion exchange resin. It enables >140 mg/mL of dynamic binding capacity in the presence of up to 150 mM NaCl, while delivering exceptional separation performance.

Comparison of resolution at different flow rates

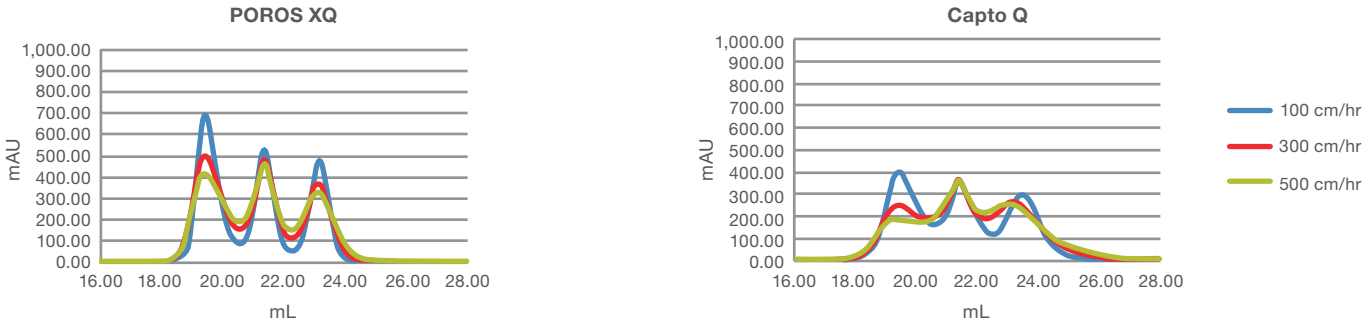


Figure 11. Comparison of resolution vs. flow rate between POROS XQ and GE Healthcare Capto™ Q resins. Three columns (0.5 cm ID x 5 cm) packed with 1 mL of either of POROS XQ or Capto Q SAX resin were loaded with a protein mixture of chicken ovalbumin, human holo-transferrin, and soybean trypsin inhibitor (3.0 mg of each protein). A gradient was applied from 0 to 1 M NaCl at a flow rate of 100 cm/hr over 20 column volumes. Purification was then repeated using elution flow rates of 300 and 500 cm/hr.

Thermo Scientific™ POROS™ HQ resin is a strong anion exchange resin that is based on a quaternized polyethyleneimine functional group yielding a high capacity, Perfusion Chromatography™ media designed for the separation and purification of biomolecules.

Comparison of resolution at different flow rates

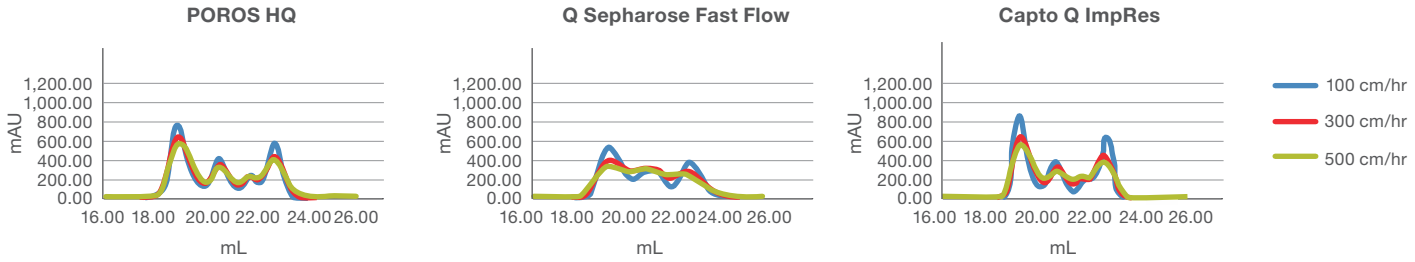


Figure 12. Comparison of resolution vs. flow rate between POROS HQ and other resins. Three columns (0.5 cm ID x 5 cm) packed with 1 mL of either of POROS HQ, GE Healthcare Capto Q ImpRes, or GE Healthcare Q Sepharose FastFlow resin were loaded with protein mixture of chicken ovalbumin, human holo-transferrin, and soybean trypsin inhibitor (3.0 mg of each protein). A gradient was applied from 0 to 1 M NaCl at a flow rate of 100 cm/hr over 20 column volumes. Purification was then repeated using elution flow rates of 300 and 500 cm/hr.

Affinity chromatography resins

Our broad menu of resins and formats enable 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 ligands result in the purification of general immunoglobulins from a crude sample. Depending on the sample source, 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.

Table 15. Antibody purification selection guide.

Mode	Description	Recommended product	Screening	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	✓			
		Protein A Plus Agarose		✓	✓	
		POROS MabCapture A Select		✓	✓	✓
		Dynabeads Protein G Magnetic Beads	✓			
		Protein G Plus Agarose		✓	✓	
		POROS MabCapture G Select		✓	✓	✓
		Pierce Protein A/G Magnetic Beads	✓			
		Protein A/G Plus Agarose		✓	✓	
		POROS MabCapture A/G Select		✓	✓	✓
		Pierce Protein L Magnetic beads	✓			
		Protein L Agarose		✓	✓	
IgG enrichment	Thiophilic adsorption	Pierce Thiophilic Adsorbent		✓	✓	
IgM enrichment	Immobilized mannan binding protein (MBP)	Immobilized Mannan Binding Protein		✓	✓	
IgA enrichment	Immobilized jacalin, a D-galactose binding lectin	Immobilized Jacalin		✓	✓	

Comparison of dynamic binding capacity at different flow rates

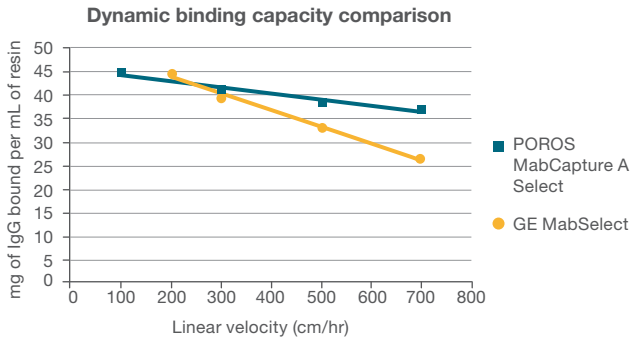


Figure 13. Comparison of dynamic binding capacity vs. flow rate. Two columns (0.46 cm ID x 20 cm) were packed with 1 mL of either of Thermo Scientific™ POROS™ MabCapture™ A Select or GE Healthcare MabSelect resin and were challenged with human IgG (5 mg/mL) at flow rates of 700, 500, 300, 200, or 100 cm/hr. The dynamic binding capacity (total protein loaded) was determined at 5% breakthrough.

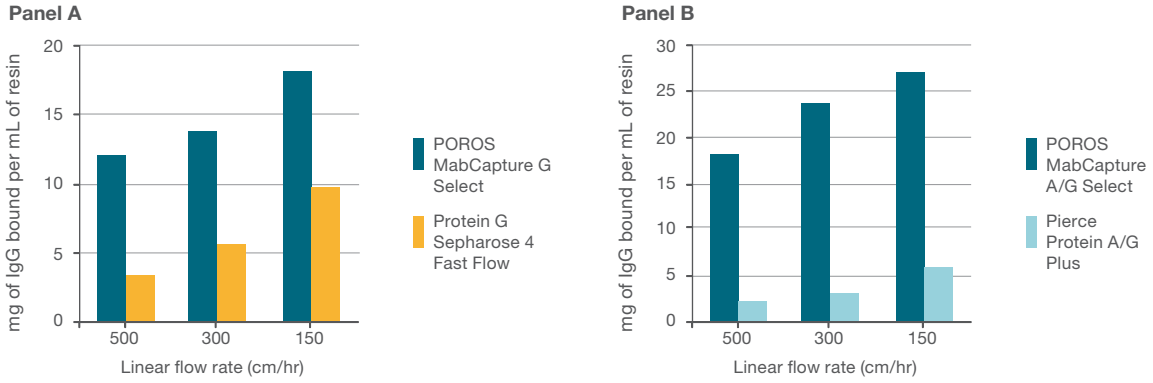


Figure 14. Comparison of dynamic binding capacity vs. flow rate. Each column (0.5 cm ID x 5 cm) was packed with 1 mL of resin and was challenged with human IgG (1 mg/mL) at flow rates of 500, 300, or 100 cm/hr (corresponding to residence times of 0.3, 1, and 2, respectively). The dynamic binding capacity (total protein loaded) was determined at 10% breakthrough. **Panel A.** Comparison between Thermo Scientific™ POROS™ MabCapture™ G Select and GE Healthcare Protein G Sepharose 4 Fast Flow resins. **Panel B.** Comparison between Thermo Scientific™ POROS™ MabCapture™ A/G Select and Pierce Protein A/G Plus resins.

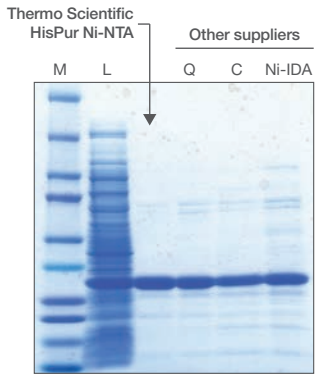
Recombinant protein purification

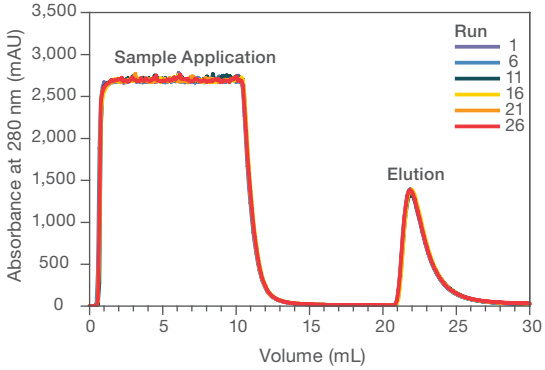
We offer a variety of Thermo Scientific™ purification resins for the purification of recombinant proteins from cultures such as *E. coli* or *Picchia*. These resins are available in multiple formats to accommodate a variety of needs, including screening, batch, pilot, and process purification. Superflow resins have undergone extensive chemical characterization.

Table 16. Recombinant protein purification selection guide.

Tag	Ligand	Features	Recommended product	Screening	Batch	Pilot	Process
6xHis	Ni-NTA	Higher protein yield	HisPur Ni-NTA Magnetic Beads	✓			
			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 beads	✓			
			Pierce Glutathione Agarose	✓	✓		
			Pierce Glutathione Superflow		✓	✓	
HA	Anti-HA	Immobilized antibody	Pierce Anti-HA Magnetic Beads	✓			
			Pierce Anti-HA Agarose		✓		
c-Myc	Anti-c-Myc	Immobilized antibody	Pierce Anti-c-Myc Magnetic Beads	✓			
			Pierce Anti-c-Myc Agarose		✓		

Comparison of protein purity and yield and resin reusability





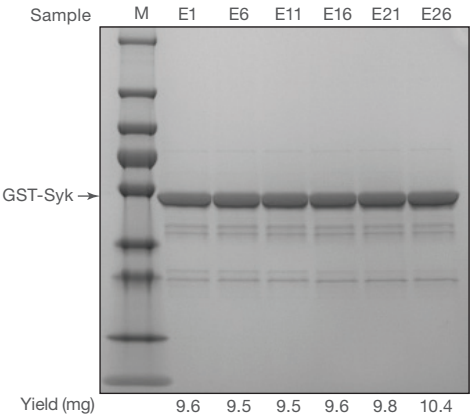


Figure 15. Thermo Scientific™ HisPur™ Ni-NTA Resin (agarose) performs as well as or better than other suppliers' nickel resins. Bacterial lysate (12 mg total protein) containing overexpressed 6xHis-GFP (green fluorescent protein) was applied to HisPur Ni-NTA Resin (Cat. No. 88221) (0.2 mL) and purified by the batch-bind method. The same amount of total protein was applied to Supplier Q (Qiagen), Supplier C (Clontech), and Ni-IDA resins per the manufacturers' instructions. Gel lanes were normalized to equivalent volume. M = molecular weight markers; L = lysate load.

Figure 16. Dependable reusability of Thermo Scientific™ Pierce™ Glutathione Superflow Agarose. Glutathione Superflow Agarose was challenged with multiple rounds of protein purification and column cleaning. An equilibrated 1 mL column (column diameter = 0.5 cm) packed with Glutathione Superflow Agarose and attached to a GE AKTA FPLC system was challenged with 10 mL of *E. coli* lysate containing overexpressed GST-Syk at a flow rate of 0.5 mL/min. After loading GST-Syk onto the column, it was washed with 10 column volumes (CV) of wash buffer followed by 10 CV of elution buffer containing 10 mM reduced glutathione. After GST-Syk protein elution, the column was treated to 5 clean-in-place cycles. One clean-in-place cycle consists of treating the column with 2 CV of 6 M guanidine-HCl, 5 CV of wash buffer, and 4 CV of 70% ethanol, followed with 5 CV of wash buffer. Purification followed by 5 clean-in-place cycles was repeated 5 times, for a total of 6 lysate challenges (cycle 1, 6, 11, 16, 21, and 26) and 25 clean-in-place treatments. GST protein yield and purity were measured by absorbance at 280 nm and the chromatogram was depicted for each of the 5 lysate challenges. Elution fractions were also analyzed by SDS-PAGE, which also revealed pure, consistent GST-Syk. M = molecular weight markers.

Biotin affinity purification

We offer a variety of Thermo Scientific™ resins for the purification of biotinylated or desthiobiotinylated proteins, peptides, and other molecules. These resins are available in multiple pack sizes, as well as in spin columns, kits, FPLC cartridges, and coated plates.

Table 17. Biotin-binding affinity resin selection guide.

Ligand	Specificity	Nonspecific binding	Elution conditions*	Recommended product	Screening	Batch	Pilot	Process
Avidin	Low	High	Harsh	Avidin Agarose Resin		✓		
Monomeric avidin	High	Low	Mild	Monomeric Avidin Resin		✓		
Streptavidin	Higher	Lower	Harsh	Pierce Streptavidin Magnetic Beads	✓			
				Streptavidin Agarose Resin		✓		
				High Capacity Streptavidin Agarose Resin		✓		
NeutrAvidin	Highest	Lowest	Harsh	NeutrAvidin Agarose Resin		✓		
				High Capacity NeutrAvidin Agarose Resin		✓		

* For specific elution conditions refer to product instructions.

Comparison of binding capacity to biotinylated BSA

Supplier	Cartridge size	Biotinylated BSA bound
Pierce High Capacity Streptavidin Chromatography Cartridge	1 mL	12.9 mg
	5 mL	75.9 mg
GE HiTrap Streptavidin HP	1 mL	10.7 mg
	5 mL	(Not offered in 5 mL size)
Pierce High Capacity NeutrAvidin Chromatography Cartridge	1 mL	12.8 mg
	5 mL	70 mg

Note: Capacity for the avidin resins was determined indirectly by subtracting the unbound biotinylated BSA present in the flow-through fractions from the total amount applied to the column.

Figure 17. Binding capacity of Thermo Scientific High Capacity Streptavidin Chromatography Cartridges is comparable to that of HiTrap columns. Columns were overloaded with biotinylated BSA and purified per manufacturer's instructions. Binding capacity was determined using the Pierce BCA Protein Assay Kit (Cat. No. 23225).

Activated supports for custom immobilization

We offer a variety of Thermo Scientific™ activated supports and accessories for the immobilization of proteins, antibodies, and other molecules. These resins or magnetic beads are available separately or in convenient kits.

Table 18. Activated support selection guide.

Target functional group	Ideal for	Recommended product	Screening	Batch	Pilot	Process
NH_2	Proteins Antibodies	Pierce NHS-Activated Magnetic Beads	✓			
		Pierce NHS-Activated Agarose		✓		
		AminoLink Plus Coupling Resin		✓	✓	
SH	Proteins Peptides Antibodies	SulfoLink Coupling Resin		✓		
CHO	Glycoproteins	GlycoLink Coupling Resin		✓	✓	
COOH	Polyclonal antibodies Unmodified peptide	CarboxyLink Coupling Resin		✓		

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

Immunoprecipitation using magnetic beads

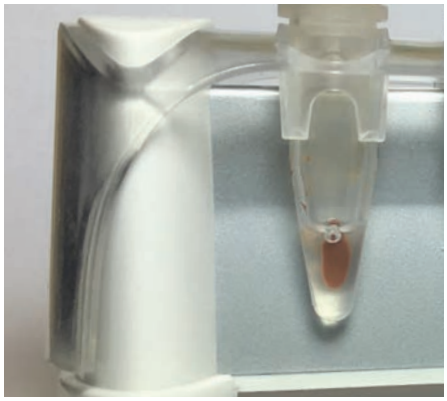
Fast and reproducible sample processing with high protein yield and low nonspecific binding

Magnetic beads have become the gold standard to use for IP and pull-down assays because they are a faster, easier, and more efficient way of pulling down the proteins than traditional Sepharose™ or agarose resins.

Thermo Fisher Scientific offers a wide variety of conjugated magnetic beads including the highly referenced Invitrogen™ Dynabeads™ magnetic beads, and also Pierce™ magnetic beads to meet most application and budget needs.

Highlights:

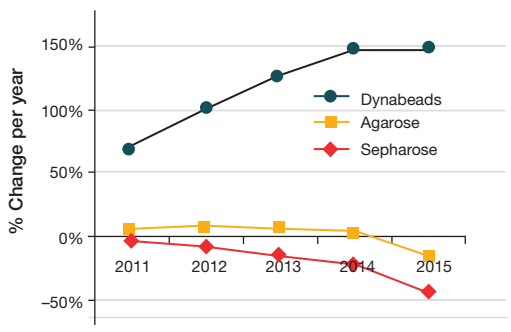
- **Low background**—little to no nonspecific binding, and no preclearing
- **Highly reproducible**—uniform beads ensure the most consistent results
- **Highly sensitive**—Dynabeads magnetic beads are the most-cited product for sensitive applications such as chromatin immunoprecipitation (ChIP) and IP of low-abundance proteins
- **Fast and easy**—Dynabeads magnetic beads offer a <40-minute IP protocol, with no centrifugation or preclearing steps



- **Antibody savings**—all binding occurs on the smooth outer surface of the beads, which conserves precious antibodies and makes for a more cost-efficient solution per sample
- **Flexible**—products for IP, co-IP, pull-down, and ChIP assays; ideal for both manual and automated protocols

Published papers on IP and ChIP

A. Immunoprecipitation—all publications



B. ChIP—*Nature* publications

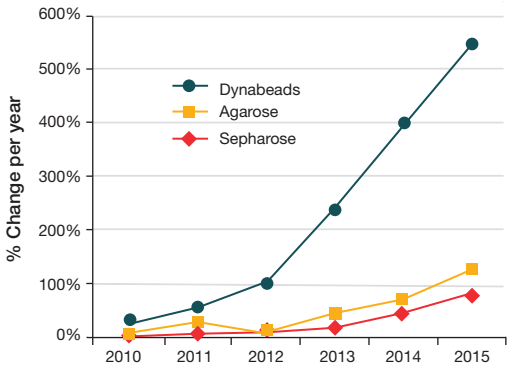
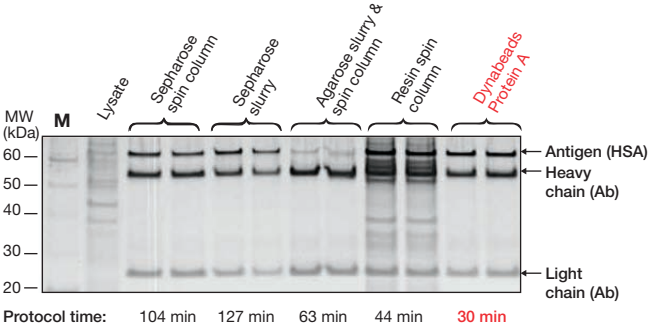


Figure 18. Published papers on immunoprecipitation (Dynabeads, agarose, or Sepharose beads). (Source: January 2016 Google Scholar)

Benchmarking vs. resin-based solutions

IP kit—Dynabeads™ Protein A vs. other suppliers

IP from Daudi cell lysate using 5 µg antibody with all products

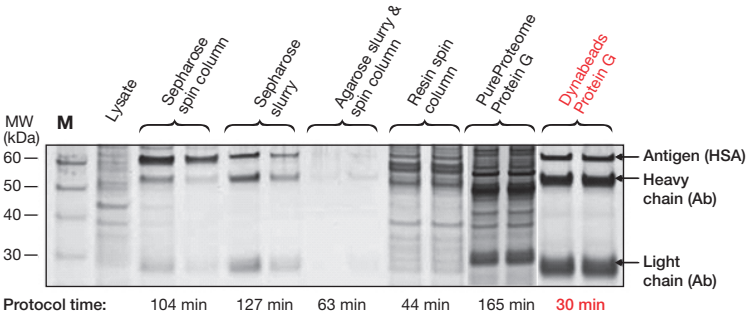


10% eluate, Novex NuPAGE 4–12% Bis-Tris gel, MES running buffer, SilverQuest Silver Staining Kit

Dynabeads IP kit—shorter protocol time. Better yield and reproducibility.

IP kit—Dynabeads™ Protein G vs. other suppliers

IP from Daudi cell lysate using 5 µg antibody with all products



10% eluate, Novex NuPAGE 4–12% Bis-Tris gel, MES running buffer, SilverQuest Silver Staining Kit

Dynabeads IP kit—shorter protocol time. Better yield and reproducibility.

Figure 19. Benchmarking Dynabeads magnetic beads against resin-based solutions. The same amount (5 µg) of antibodies (Ab) and cell lysates were used for all IP protocols. All the antibodies on the bead surface are accessible for optimal, highly reproducible antigen binding. Results show shorter protocol time and better yields with Dynabeads magnetic beads vs. alternate resin solutions.

Benchmarking vs. other magnetic-based solutions

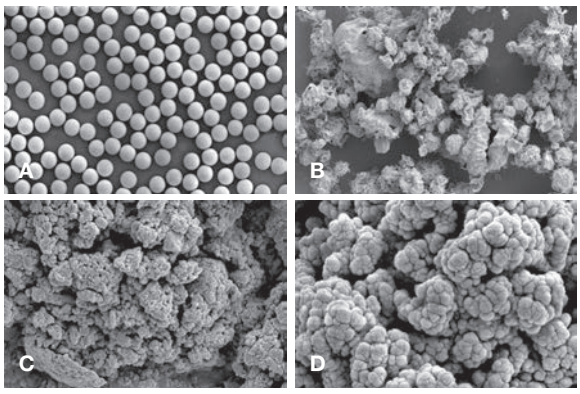


Figure 20. The magnetic bead you choose will affect your results. Dynabeads magnetic beads have a defined outer surface for protein binding, with no inner surface to trap any unwanted proteins.

A. Dynabeads magnetic beads are the most uniform, monodisperse superparamagnetic beads, manufactured with highly controlled product qualities to ensure the highest degree of reproducibility.

B–D. Magnetic particles from alternative suppliers have variable shapes and sizes that trap impurities, resulting in lower reproducibility and increased nonspecifi binding.

Yield

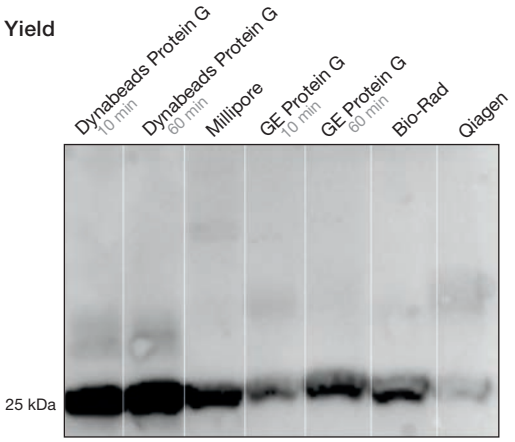


Figure 21. Protein yield results using western blotting. Dynabeads Protein G magnetic beads have the best overall performance in yield, capacity, and nonspecific binding.

Nonspecific binding

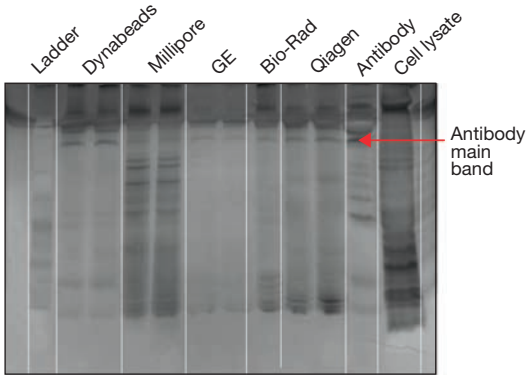


Figure 22. Nonspecific binding results using silver staining. Dynabeads Protein G magnetic beads show very little nonspecific binding, and provide the best signal-to-noise ratio.

Table 19. Choose your isolation strategy and find your product.

Choose this if you have	Surface coating on the magnetic beads	Type of ligand required	Mass spec compatible	Non-specific binding	IP protocol time	Main benefits for IP	Products
Protein-specific antibody	Protein A, G, or L	Primary antibodies from most species. Protein A, G, and L bind different antibody species and subclasses with different specificities	No	Low	Dynabeads: <40 minutes Pierce beads: 130–180 minutes	Dynabeads—fastest, easiest protocol with low nonspecific binding and high yield and reproducibility	Dynabeads Protein A Dynabeads Protein G Dynabeads Protein A Immunoprecipitation Kit Dynabeads Protein G Immunoprecipitation Kit Pierce Protein A/G Magnetic Beads Pierce Protein L Magnetic Beads
	Secondary antibodies	Mouse IgG or rabbit IgG	No†	Low	Dynabeads: <40 minutes	• 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-activated beads*	Any protein ligand (e.g., antibody, peptide)	Yes	Ultralow	Dynabeads: Ab coupling time: over-night; co-IP protocol time: 30–40 minutes	• 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
Biotinylated antibody	Streptavidin	Any biotinylated antibody or ligand	Yes	Low	30–40 minutes	• Binds any biotinylated protein • For samples high in soluble IgGs • Recombinant Ab lacking the Fc-region	Dynabeads M-280 Streptavidin Dynabeads M-270 Streptavidin Dynabeads MyOne Streptavidin C1 Dynabeads MyOne Streptavidin T1
Recombinant protein	Fusion tags	Different beads bind proteins with the following tags: His, GST, HA, c-Myc	Yes	Low	Dynabeads His-tag beads: ~25 minutes Pierce beads: ~70 minutes	• Purify many different proteins incorporated with the same tag • No need for antibodies	See **thermofisher.com/iptags** for product overview

* See more choices in surface-activated Dynabeads products for the binding and capture of additional targets.
† Contains Tween™-20 detergent that is contaminating for the mass spectrometry.

Choose if you have an antibody that recognizes your protein—your choice of antibody-binding products depends on whether your downstream assay is mass spectrometry, or if you don't want the antibody co-eluted with your target protein.

Antibody binding is the most common method and is used when your target antibody can be bound directly to the beads or indirectly to a precoated ligand on the magnetic beads.

Choose if you have a biotinylated antibody that recognizes your protein—your best choice when using a biotinylated antibody with streptavidin-coated beads for IP:

- If you have a sample rich in soluble IgGs
- If you have a recombinant antibody lacking Fc regions
- If you need a bead compatible with mass spectrometry (secondary-coated and epoxy-coated Dynabeads products are also compatible with mass spectrometry)

Choose if you have a recombinant protein (fusion tag)—the most popular fusion tags for recombinant protein expression are covered by Pierce and Dynabeads products. These include His tag, GST tag, HA tag, and c-Myc tag. See **thermofisher.com/iptags** for product list.

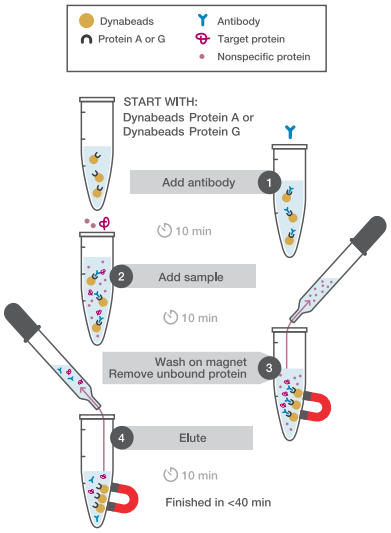


Figure 23. Immunoprecipitation in <40 minutes. Dynabeads magnetic beads precoupled with Protein A or Protein G act as a suspendable solid support that can be fixed by the use of a magnet. This allows for simple and efficient antibody capture, followed by immunoprecipitation of your pure target peptides, proteins, protein complexes, or other antigens.

Co-IP—with Dynabeads magnetic beads, you skip unnecessary steps and help ensure intact protein complexes

If you are using techniques such as Sepharose beads and spin columns for pull-down, your protein complexes can dissociate from exposure to large surfaces, mechanical strain (e.g., centrifugation), dilution, and excessive handling (i.e., preclearing). To preserve native protein conformations and large protein complexes, use Invitrogen™ Dynabeads™ Co-Immunoprecipitation Kit. Just couple your antibody directly to the Dynabeads magnetic beads, add the sample containing the target protein and use the magnet to separate your protein complexes.

Advantages of Dynabeads magnetic beads for protein complex isolation:

- Quick and easy pull-down of intact, functional protein complexes
- No time-consuming preparation steps
- Only isolate the proteins you want
- Can be adapted for high-throughput applications
- Antibody is covalently bound to the bead, thus no crosslinking required

"Dynabeads are absolutely the best technology we have found so far for pulling out large complexes."

—Dr. Michael P. Rout, Rockefeller University

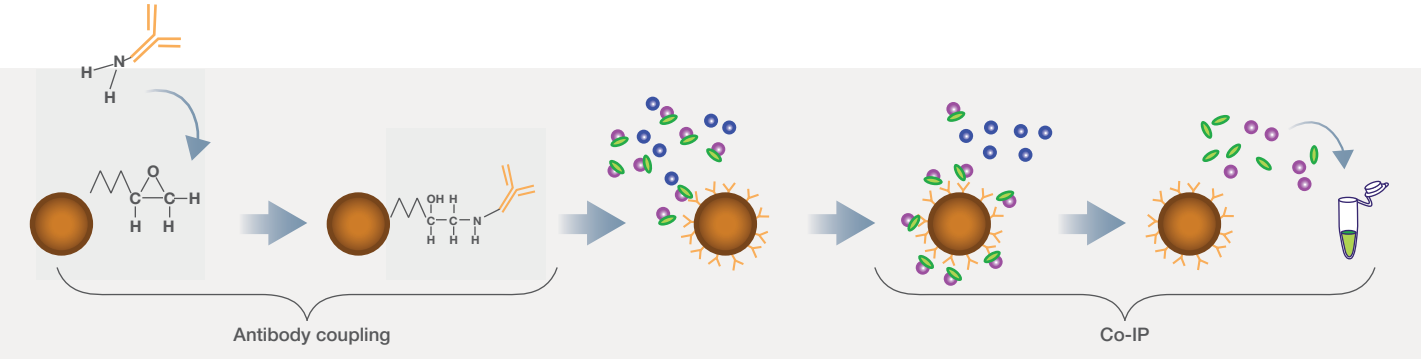


Figure 26. Dynabeads™ Co-Immunoprecipitation Kit (Cat. No. 14321D). The co-IP is performed in several steps. First, the antibody is covalently coupled to the beads. Then the antibody-coupled beads are added to the sample to bind to the target protein complex, and washed/purifi d using a DynaMag™ Magnet.

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

Ordering information

Product	Quantity	Cat. No.
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 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
B-PER Complete Bacterial Protein Extraction Reagent	250 mL	89821
To view additional pack sizes and products, go to **thermofisher.com/proteinextraction**		
Inhibitor cocktails and tablets		
Halt Protease Inhibitor Cocktail (100X)	1 mL	87786
Halt Protease Inhibitor Cocktail (100X), EDTA-Free	1 mL	87785
Pierce Protease Inhibitor Mini Tablets	30 tablets	88665
Pierce Protease Inhibitor Tablets	20 tablets	88265
Pierce Protease Inhibitor Mini Tablets, EDTA-free	30 tablets	88666
Pierce Protease Inhibitor Tablets, EDTA-free	20 tablets	88266
Halt Phosphatase Inhibitor Cocktail (100X)	1 mL	78420
Pierce Phosphatase Inhibitor Mini Tablets	20 tablets	88667
Halt Protease and Phosphatase Inhibitor Cocktail (100X)	1 mL	78440
Halt Protease and Phosphatase Inhibitor Cocktail (100X), EDTA-Free	1 mL	78441
Pierce Protease and Phosphatase Inhibitor Mini Tablets	30 tablets	88668
Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-free	30 tablets	88669
To view additional pack sizes and products, go to **thermofisher.com/inhibitorcocktails**		
Detergents		
Tween-20 Surfact-Amps Detergent Solution	6 x 10 mL	28320
Tween-20 Surfact-Amps Detergent Solution	50 mL	85113
Tween-80 Surfact-Amps Detergent Solution	6 x 10 mL	28328
Tween-80 Surfact-Amps Detergent Solution	50 mL	28329
Triton X-100 Surfact-Amps Detergent Solution	6 x 10 mL	28314
Triton X-100 Surfact-Amps Detergent Solution	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
NP-40 Surfact-Amps Detergent Solution	50 mL	85124
Brij-35 Surfact-Amps Detergent Solution	6 x 10 mL	28316
Brij-35 Surfact-Amps Detergent Solution	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.
Dialysis devices, cassettes, and flasks		
Slide-A-Lyzer MINI Dialysis Devices, 10K MWCO, 0.1 mL	50 devices	69570
Slide-A-Lyzer MINI Dialysis Devices, 10K MWCO, 0.5 mL	25 devices	88401
Slide-A-Lyzer MINI Dialysis Devices, 10K MWCO, 2 mL	25 devices	88404
Slide-A-Lyzer G2 Dialysis Cassettes, 7K MWCO, 0.5 mL	10 cassettes	87727
Slide-A-Lyzer G2 Dialysis Cassettes, 7K MWCO, 3 mL	10 cassettes	87728
Slide-A-Lyzer G2 Dialysis Cassettes, 0.5K MWCO, 0.5 mL	8 cassettes	87729
Slide-A-Lyzer G2 Dialysis Cassettes, 3K MWCO, 3 mL	6 cassettes	87730
Slide-A-Lyzer G2 Dialysis Cassettes, 15K MWCO, 15 mL	6 cassettes	87731
Slide-A-Lyzer G2 Dialysis Flask, 10K MWCO, 250 mL	4 flasks	87762
To view additional pack sizes and MWCOs, go to **thermofisher.com/dialysis**		
Desalting products		
Zeba 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, 7K MWCO, 2 mL	25 columns	89890
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 Chromatography Cartridges, 7K MWCO, 1 mL	5 cartridges	89934
Zeba Chromatography Cartridges, 7K MWCO, 5 mL	5 cartridges	89935
Zeba 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, 10K MWCO, 0.5 mL	25/pkg	88513
Pierce Protein Concentrator PES, 10K MWCO, 2–6 mL	24/pkg	88517
Pierce Protein Concentrator PES, 10K MWCO, 5–20 mL	24/pkg	88528
Pierce Protein Concentrator PES, 10K MWCO, 20–100 mL	4/pkg	88535
To view additional pack sizes and MWCOs, go to **thermofisher.com/concentrators**		

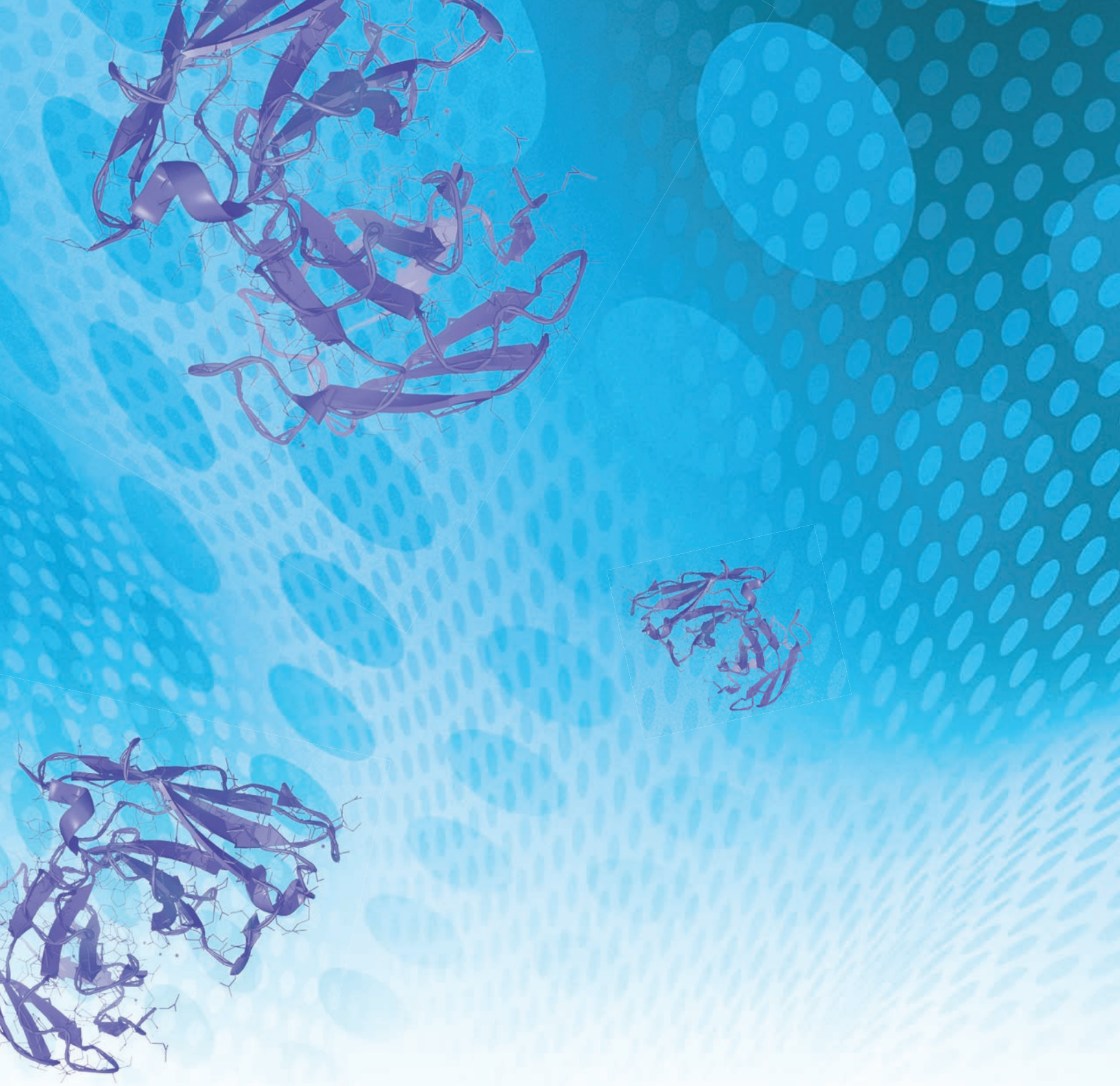
Ordering information

Product	Quantity	Cat. No.
Strong cation exchange purification esins		
POROS XS Resin	10 ml	82071
Strong anion exchange purification esins		
POROS XQ Resin	10 ml	82073
POROS HQ Resin	10 ml	82077
Antibody purification esins		
Protein A Plus Agarose	5 mL	22811
POROS MabCapture A Select	15 mL	82080
Protein G Plus Agarose	2 mL	22851
POROS MabCapture G Select	15 mL	82083
Protein A/G Plus Agarose	2 mL	20423
POROS MabCapture A/G Select	15 mL	82086
Protein L Agarose	2 mL	20510
Melon Gel Monoclonal IgG Purification Ki	Kit	45214
Recombinant protein purification esins and magnetic beads		
HisPur Ni-NTA Magnetic Beads	2 mL	88831
HisPur Ni-NTA Agarose Resin	10 mL	88221
HisPur Ni-NTA Superflow Aga ose	10 mL	25214
HisPur Cobalt Agarose Resin	10 mL	89964
HisPur Cobalt Superflow Aga ose	10 mL	25228
Pierce Glutathione Magnetic Beads	4 mL	88821
Pierce Glutathione Agarose	10 mL	16100
Pierce Glutathione Superflow Aga ose	10 mL	25236
Pierce Anti-c-Myc Agarose	2 mL	20168
Pierce Anti-HA Agarose	1 mL	26181
Biotin binding purification esins and magnetic beads		
Pierce Streptavidin Magnetic Beads	1 mL	88817
High Capacity Streptavidin Agarose Resin	2 mL	20357
High Capacity NeutrAvidin Agarose Resin	5 mL	29202
Monomeric Avidin Agarose Resin	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 M-280 Tosylactivated	2 mL	14203
Dynabeads MyOne Tosylactivated	2 mL	65501
Dynabeads M-270 Carboxylic Acid	2 mL	14305D
Dynabeads MyOne Carboxylic Acid	2 mL	65011
Dynabeads M-270 Amine	2 mL	14307D
Pierce NHS-Activated Agarose, Dry	1 g	26196
AminoLink Plus Coupling Resin	10 mL	20501
GlycoLink Immobilization Kit	10 columns	88941
SulfoLink Coupling Resin	10 mL	20401
CarboxyLink Coupling Resin	25 mL	20266

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

Product	Quantity	Cat. No.
Immunoprecipitation using magnetic beads		
Dynabeads Protein A	1 mL	10001D
Dynabeads Protein G	1 mL	10003D
Dynabeads Protein A Immunoprecipitation Kit	2 mL	10006D
Dynabeads Protein G Immunoprecipitation Kit	2 mL	10007D
Pierce Protein A/G Magnetic Beads	1 mL	88802
Pierce Protein L Magnetic Beads	1 mL	88849
Dynabeads Antibody Coupling Kit	1 kit	14311D
Dynabeads Co-Immunoprecipitation Kit	40 reactions	14321D
Dynabeads His-Tag Isolation and Pulldown	2 mL	10103D
Dynabeads M-280 Sheep Anti-Mouse IgG	2 mL	11201D
Dynabeads M-280 Sheep Anti-Rabbit IgG	2 mL	11203D
Dynabeads M-280 Streptavidin	2 mL	60210
Dynabeads M-270 Streptavidin	2 mL	65305
Dynabeads MyOne Streptavidin C1	2 mL	65001
Dynabeads MyOne Streptavidin T1	2 mL	65602

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



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

fisherscientific

In the United States:
For customer service, call 1-800-766-7000
To fax an order, use 1-800-926-1166
To order online: fishersci.com

In Canada:
For customer service, call 1-800-234-7437
To fax an order, use 1-800-463-2996
To order online: fishersci.ca

ThermoFisher SCIENTIFIC