Protein preparation handbook

Cell lysis | Subcellular fractionation | Protease and phosphatase inhibition | Dialysis | Desalting | Concentration | Purification | Immunoprecipitation | Co-immunoprecipitation | Pull-down

ThermoFisher SCIENTIFIC

Extract. Clean up. Purify. Immunoprecipitate.

We offer a full range of optimized tools for efficient protein extraction, fractionation and 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 cleanup of proteins and antibodies for downstream applications.

Protein extraction

Protein extraction techniques vary depending on the source of the starting material, the location of the protein of interest within the cell, 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 yields 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 present in unpurified detergents.
- **Protein stabilization**
  Cell lysis disrupts cell membranes and organelles, resulting in unregulated enzymatic 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 that can inactivate or block the activities of proteases and phosphatases. Thermo Scientific™ Halt™ and Thermo Scientific™ Pierce™ Protease and Phosphatase Inhibitor Cocktails Tablets and Capsules are broad-spectrum blends in both liquid (100X), solid formats for complete protein protection during extraction.

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 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 migration of small contaminants to slow 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 are specifically designed to provide consistent performance over a wide range of protein concentrations and sample sizes. High recovery of protein 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 purification

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, IEX 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 IEX 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. Since 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 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 IP, co-IP, and pull-down products 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

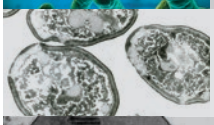
Gentle formulations designed to maximize protein yield and activity

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**—only produces 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 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	

Comparison of cross-contamination between subcellular fractions

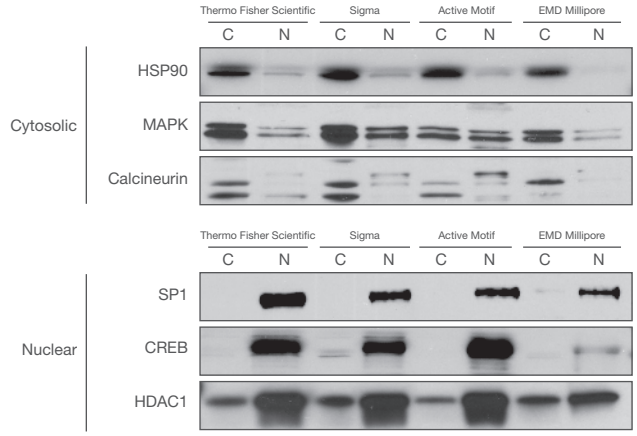


Figure 1. Nuclear and cytosolic fractions are obtained with minimal cross-contamination. HeLa cells were extracted with the Thermo Scientific™ NE-PER™ Nuclear and Cytoplasmic Extraction Reagents or with nuclear extraction kits from other vendors. Samples of the nuclear and cytosolic fractions were analyzed 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

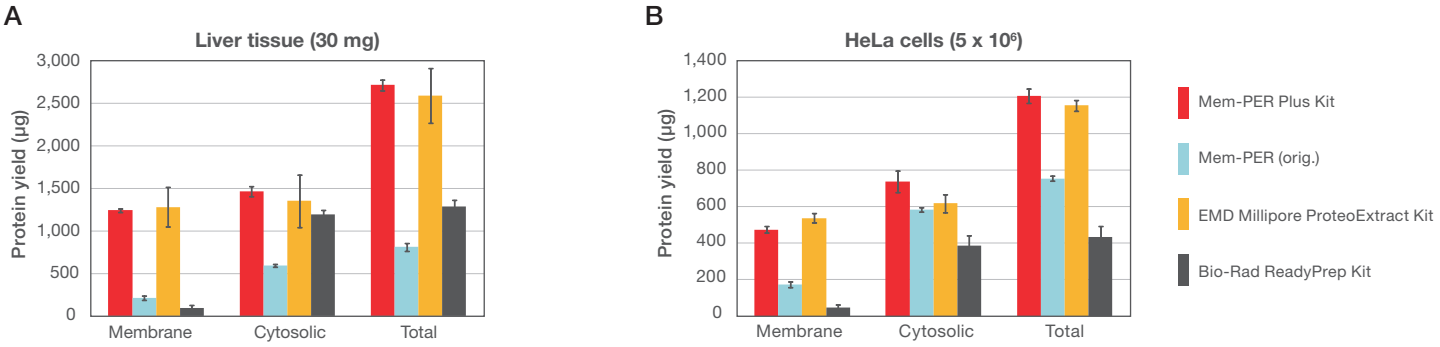


Figure 2. Improved protein yield using the Thermo Scientific™ Mem-PER™ Plus Membrane Protein Extraction Kit (Cat. No. 89842). Membrane proteins were isolated from mouse liver tissue and HeLa cells using four commercial extraction kits. Protein yields (µg) for membrane, cytosolic, and total fractions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

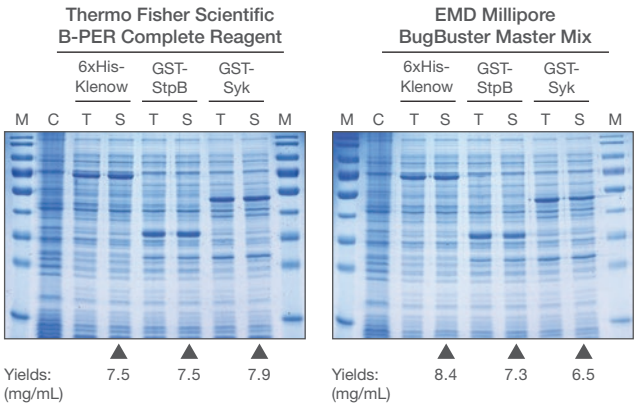


Figure 3. Protein yield comparison of two bacterial cell lysis reagents. *E. coli* ER2566/pLATE51-Klenow, ER2566/pGST-CC-StpB, and ER2566/pGS-Syk cell pellets (0.5 g), were resuspended in 2.5 mL aliquots of Thermo Scientific™ B-PER™ Complete Bacterial Protein Extraction Reagent (Cat. No. 89821) or EMD Chemicals 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.

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

Detergents

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

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 provide high purity, quality, and stability. Unlike neat (undiluted) detergents, which are extremely viscous, Surfact-Amps 10% solutions are easy to pipet 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.

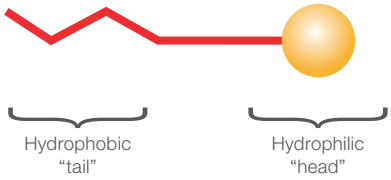


Figure 4. Generic structure of a detergent molecule.

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
Octylglucoside	Nonionic	27	8,000	292	23–25	0.6716–0.7300	>100	Yes
Octylthioglucoside	Nonionic	—	—	308	9	0.2772	>100	Yes
SDS	Anionic	62	18,000	288	6–8	0.1728–0.2304	>100	Yes
CHAPS	Zwitterionic	10	6,149	615	8–10	0.4920–0.6150	>100	Yes

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

Protease and phosphatase inhibitors

Broad-spectrum formulations for complete protein protection

Protease and phosphatase inhibitor cocktails, tablets, or capsules are ideal for the protection of proteins during extraction and lysate preparation from primary cells, cultured mammalian cells, animal tissues, plant tissues, yeast cells, and bacterial cells. Formulations are packaged in multiple sizes, and EDTA-free versions are available for assays that are sensitive to divalent cations. The Pierce inhibitor tablets and capsule contents are formulated to dissolve quickly into a clear solution, and are fully compatible with all Pierce protein assays.

Highlights:

- **Convenient**—ready-to-use, fully disclosed, broad-spectrum formulations available as either liquid cocktails, tablets, or capsules, in multiple pack sizes and with a minimum of 1 year of shelf life
- **Complete protection**—all-in-one formulations containing both protease and phosphatase inhibitors are offered in both liquid and tablet formulations (with EDTA or EDTA-free)
- **Compatible**—use directly with Thermo Scientific™ Pierce™ Cell Lysis Buffers, other commercial, or homemade detergent-based lysis reagents

Table 3. Components present in Halt inhibitor cocktails and Pierce inhibitor tablets and capsules.

Inhibitor component	Target (mechanism)	Protease liquid cocktails and tablets	Protease capsules	Phosphatase liquid cocktails and tablets	Combined protease and phosphatase liquid cocktails and tablets
AEBSF·HCl	Serine proteases (irreversible)	•	•		
Aprotinin	Serine proteases (reversible)	•			•
Bestatin	Aminopeptidases (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 phosphatases			•	•
Sodium pyrophosphate	Serine/threonine phosphatases			•	•

* EDTA not in EDTA-free formulations.

Comparison of protease and phosphatase inhibition

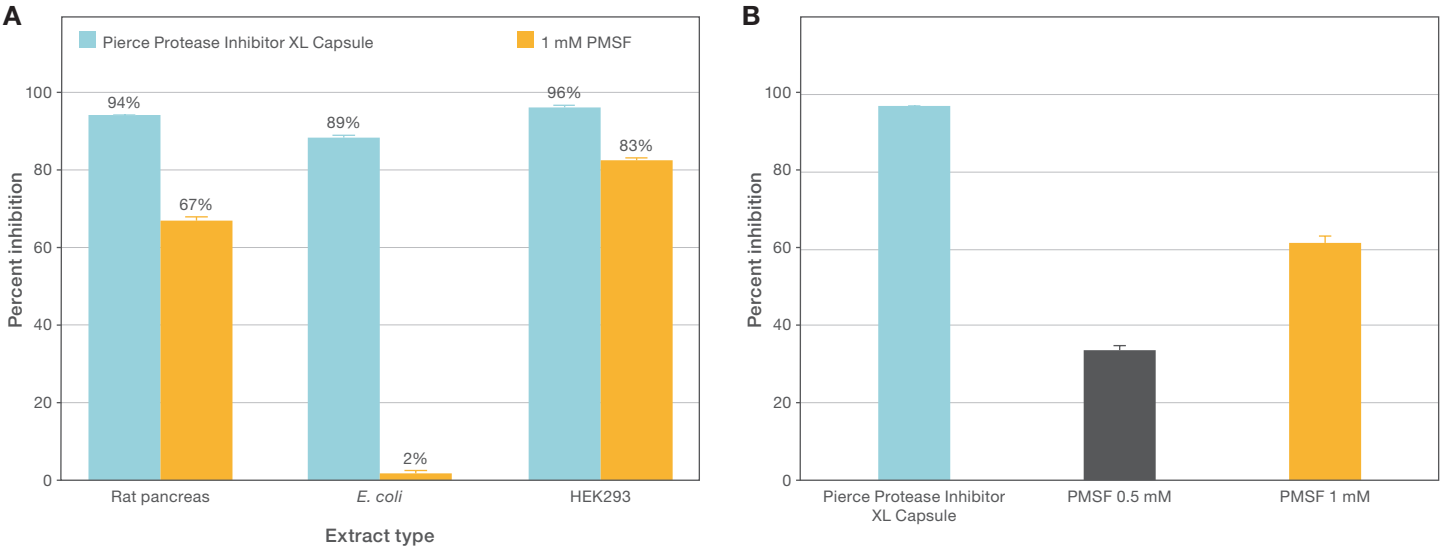


Figure 5. Performance comparison between Thermo Scientific Pierce Protease Inhibitor XL Capsule and PMSF. (A) Pancreatic extract (100 µL; 0.5 µg/µL), *E. coli* extract (100 µL; 0.5 µg/µL), and HEK293 extract (100 µL; 0.25 µg/µL) were incubated with a quenched fluorescent trypsin-cleavable substrate in the presence of Pierce Protease Inhibitor XL Capsules or 1 mM PMSF. Reactions were incubated for 1 hr at 37°C, fluorescence was determined at the appropriate emission wavelength, and percent protease inhibition is shown. **(B)** Pancreatic extract (100 µL; 0.5 µg/µL) was incubated with a quenched fluorescent protease-cleavable substrate (Invitrogen™ EnzChek™ Protease Assay Kit, red fluorescence) in the presence of Pierce Protease Inhibitor XL Capsules, or 0.5 mM or 1 mM PMSF. Reactions were incubated for 18 hr at 37°C, fluorescence was determined at the appropriate emission wavelength, and percent inhibition is shown.

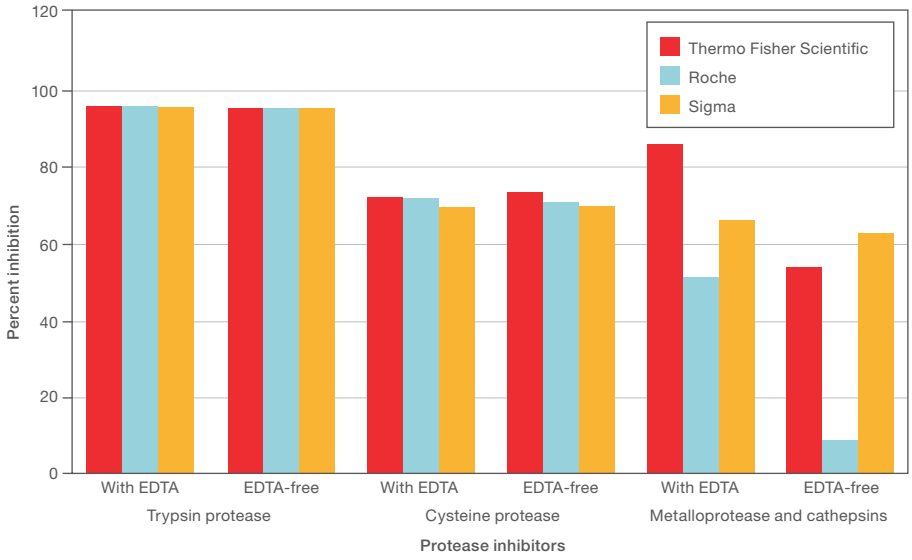


Figure 6. Performance comparison between three commercially available protease inhibitor tablets. Pancreatic extract (100 µL; 0.5 µg/µL) was incubated with quenched fluorescent protease-cleavable substrates for trypsin, cysteine, and metalloprotease and cathepsins, in the presence of the reformulated Thermo Scientific™ Pierce™ Protease Inhibitor Mini Tablets, Roche™ cOmplete™ Protease Inhibitor Tablets, and Sigma-Aldrich™ SIGMAFAST™ Protease Inhibitor Cocktail Tablets, with and without EDTA. Reactions were incubated for 1 hr at 37°C, and fluorescence was determined at the appropriate emission wavelengths. The percent inhibition is shown for each protease inhibitor formulation.

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

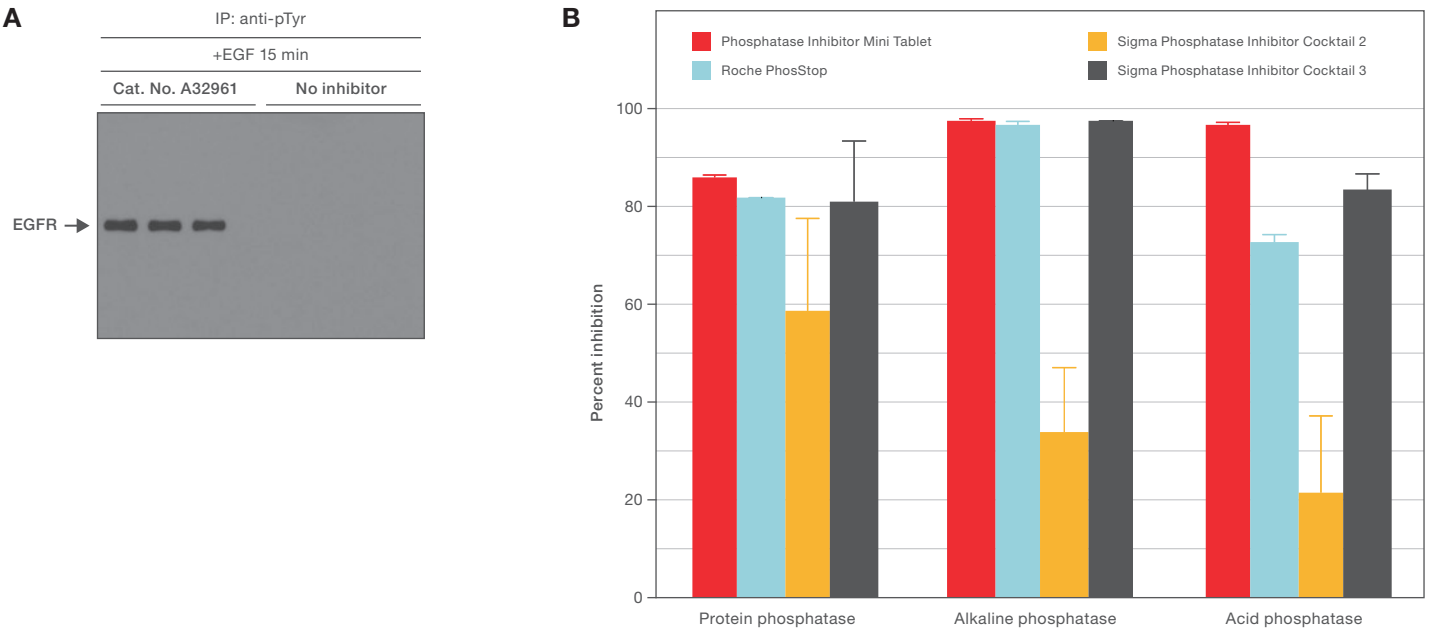
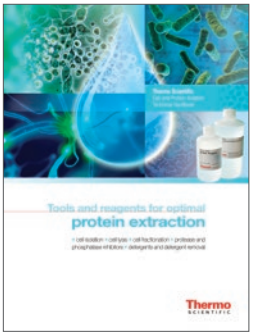


Figure 7. Protein phosphorylation is preserved in cell extracts. (A) HCT116 cells were serum-starved, then either treated with EGF for 15 min or left as control cells. Cell lysates were prepared in Thermo Scientific™ Pierce™ IP Lysis Buffer (Cat. No. 87788) with Thermo Scientific™ Protease and Phosphatase Inhibitor Mini Tablets, EDTA-Free (Cat. No. A32961), or with no inhibitor. Lysate containing 500 µg of protein was then incubated with 5 µg of phospho-tyrosine antibody overnight at 4°C. The complex was then incubated with Thermo Scientific™ Pierce™ Protein A/G Magnetic Beads for 1 hr at room temperature. Beads were washed, and low-pH elution was performed. The eluates were subjected to western blotting, and the membrane was then probed with EGFR antibody for chemiluminescence detection. **(B)** The degree of inhibition for protein, alkaline, and acid phosphatase activity was determined in kidney extract (25 µL; 0.5 µg/µL) by incubating extracts with a fluorogenic substrate (MFP or FDP) that measures phosphatase activity upon desphosphorylation in the presence of Pierce Phosphatase Inhibitor Mini Tablets, Roche™ PhosStop™ Phosphatase Inhibitor Tablets, and Sigma-Aldrich™ Phosphatase Inhibitor Cocktail 2 and 3 liquid formulations. Reactions were incubated for 1 hr at 37°C, and fluorescence was determined at the appropriate emission wavelength. The percent inhibition is shown for each phosphatase inhibitor formulation.

Thermo Scientific™ benchtop centrifuges deliver efficient sample processing in cell culture applications, spin column and microplate processing, and a variety of separation needs. In addition, the capacity and ergonomic features of our centrifuges are of exceptional value for everyday sample preparation.

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



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 total protein extraction or subcellular fractionation reagents. Preserve protein structure and function by utilizing our protease and phosphatase inhibitor cocktails and tablets. Improve your protein biology methods with our highly purified and precisely diluted detergent solutions.

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

Slide-A-Lyzer dialysis products

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

Thermo Scientific™ dialysis units 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 recovery**—low-binding plastics and membranes help minimize sample loss compared to filtration and resin systems
- **Convenient**—easy-to-grip design 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 4. 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

* MWCO: Molecular weight cut off.

Protein recovery by molecular weight cutoff (MWCO)

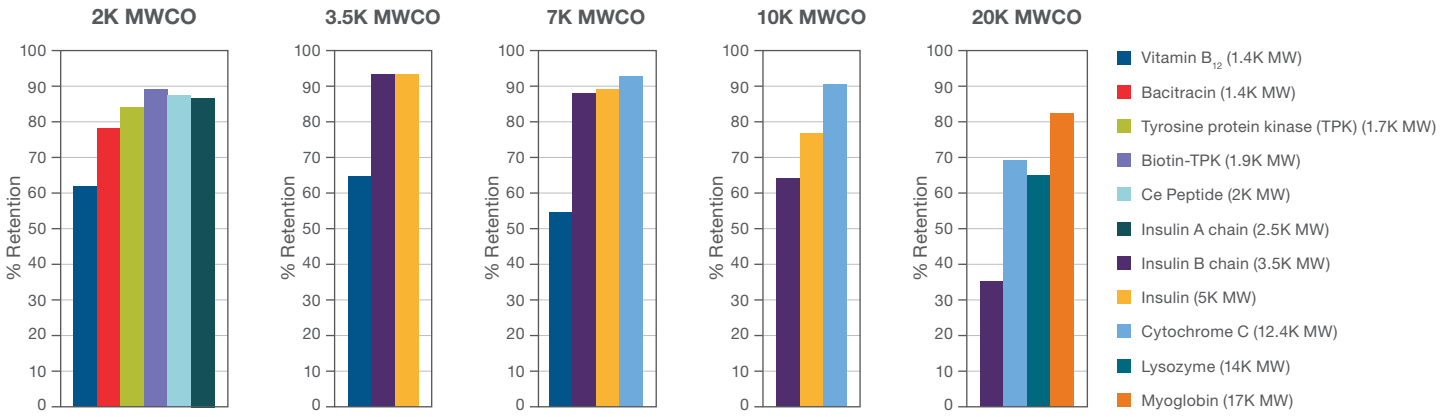


Figure 8. Sample retention by the 2K, 3.5K, 7K, 10K, and 20K MWCO Thermo Scientific™ Slide-A-Lyzer™ cassette membrane. Individual proteins or vitamin B_{12} (1 mg/mL) in either saline or 0.2 M carbonate-bicarbonate buffer, pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of retentate was estimated using either the Pierce BCA Protein Assay Kit or absorption at 360 nm (for vitamin B_{12}).

Dialysis rates for various formats

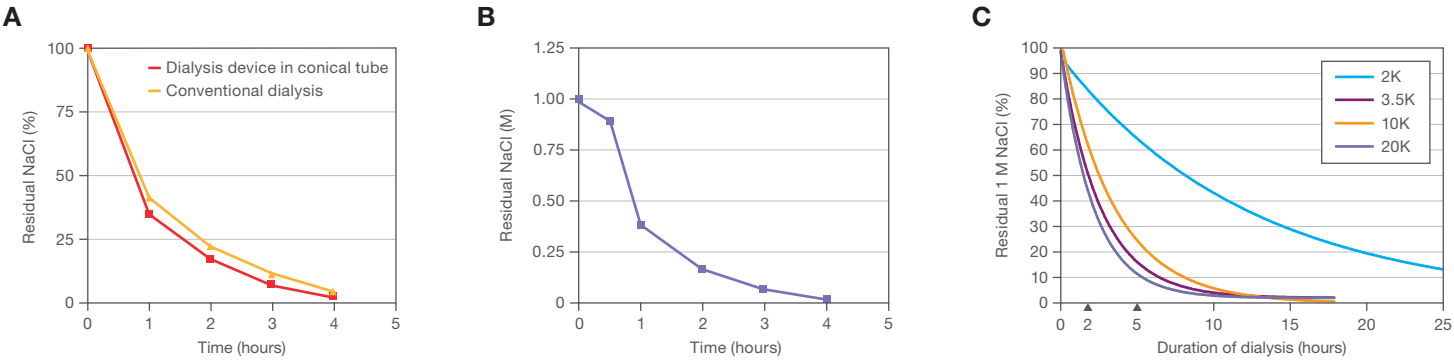


Figure 9. The rate of removal of NaCl using various dialysis products. NaCl removal from samples was determined by measuring the conductivity of the retentate at the indicated times. **(A)** Slide-A-Lyzer MINI Dialysis Device (10K MWCO, 2 mL) versus conventional dialysis. Bovine serum albumin (BSA) samples (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. **(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. **(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 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 5. Zeba desalting products selection guide by format and recommended sample volume.

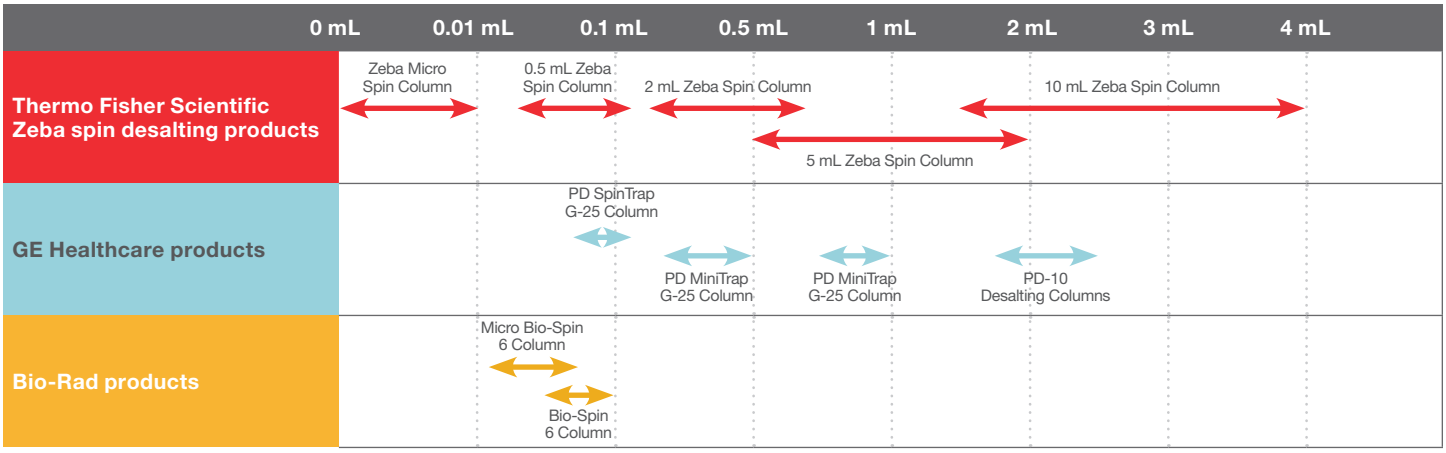
Type	Spin columns					Spin plate	Chromatography columns	
Format	**Micro**	**0.5 mL**	**2 mL**	**5 mL**	**10 mL**	**96-well**	**1 mL**	**5 mL**
								
Resin bed	75 µL	0.5 mL	2 mL	5 mL	10 mL	550 µL	1 mL	5 mL
Sample volume (7K MWCO)	2–12 µL	30–130 µL	200–700 µL	500–2,000 µL	700–4,000 µL	20–100 µL	50–250 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 6. 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 = Not recovered

Table 7. Comparison of recommended sample-volume capacity of common spin desalting products.



Comparison of protein recovery and sample dilution

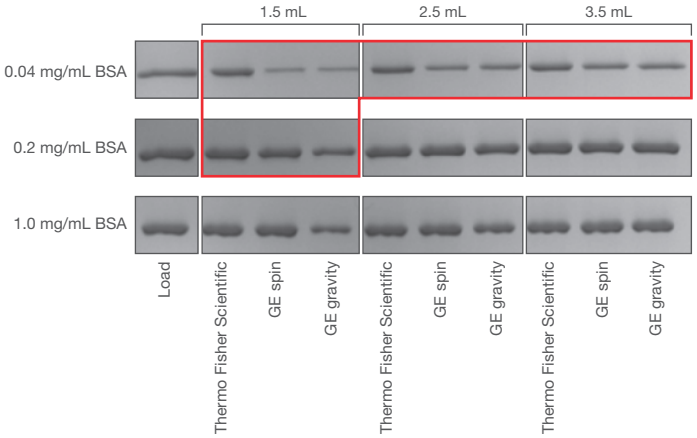


Figure 10. Zeba Spin Desalting Columns result in a high protein recovery while providing minimal sample dilution over a wider range of sample concentrations and volumes compared to alternative products. Zeba Spin Desalting Columns, 10 mL (7K MWCO) (Cat. No. 89893) and GE PD-10 Columns were used to desalt 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 Protein 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 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 8. 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 MWCO

Protein recovery compared to other suppliers

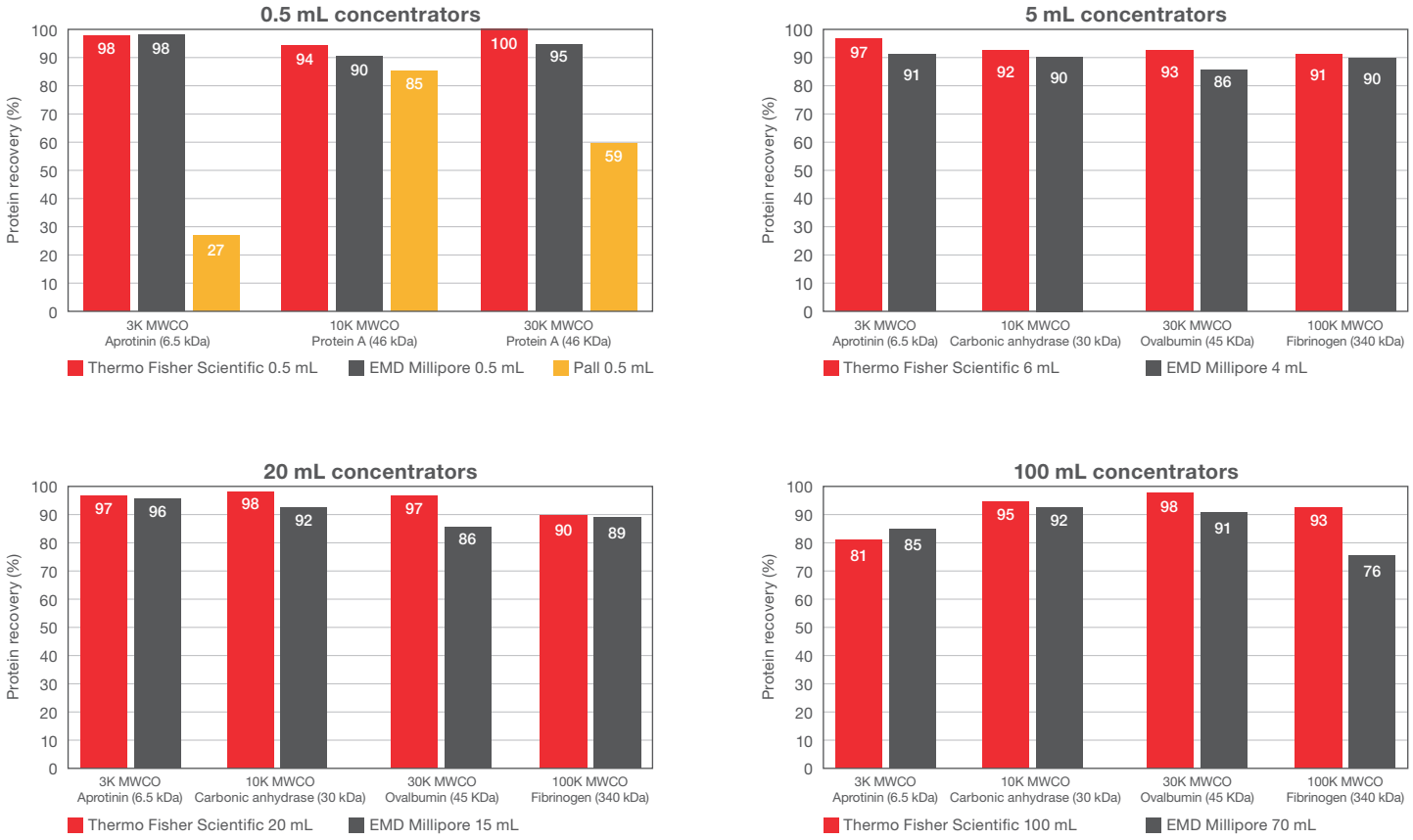
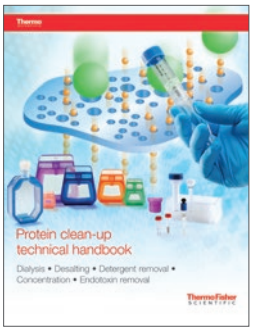


Figure 11. Comparison of protein recovery between Pierce Protein Concentrators (using 3K, 5K, 10K, 30K, or 100K MWCO) and other vendors for 0.5 mL, 6 mL, 20 mL, and 100 mL concentrators. Samples of different protein solutions were centrifuged in Pierce Protein Concentrators and other suppliers' concentrators according to manufacturers' 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, 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 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 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 multistep 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 activated support. Accessory products are available for increased convenience, including disposable columns and binding and elution buffers. Rapid protein 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 resins, 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 other leading suppliers

Table 9. 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 10. 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 superior physical and chemical stability, allowing easier handling and packing. These high-capacity chromatography media are designed to deliver excellent separation and scale-up capabilities.

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 11. 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			•	•	•

Strong anion exchange (SAX) resins and spin columns

Thermo Scientific™ POROS™ HQ and XQ resins are strong anion exchange resins that are based on a quaternized polyethyleneimine functional group. The POROS XQ resin is a next-generation, high-capacity, high-resolution, salt-tolerant strong anion exchange resin, providing >140 mg/mL in up to 150 mM NaCl, while delivering exceptional separation performance.

The high dynamic binding capacity enables reduced column size, a smaller footprint, decreased water and buffer usage, and reduced cycling. The low operating back pressure and linear pressure versus flow responses drive flexible scalability.

The Pierce Strong Anion Exchange Spin columns are ideal for processing samples of 0.5 mL to 20 mL volumes.

Comparison of resolution at different flow rates

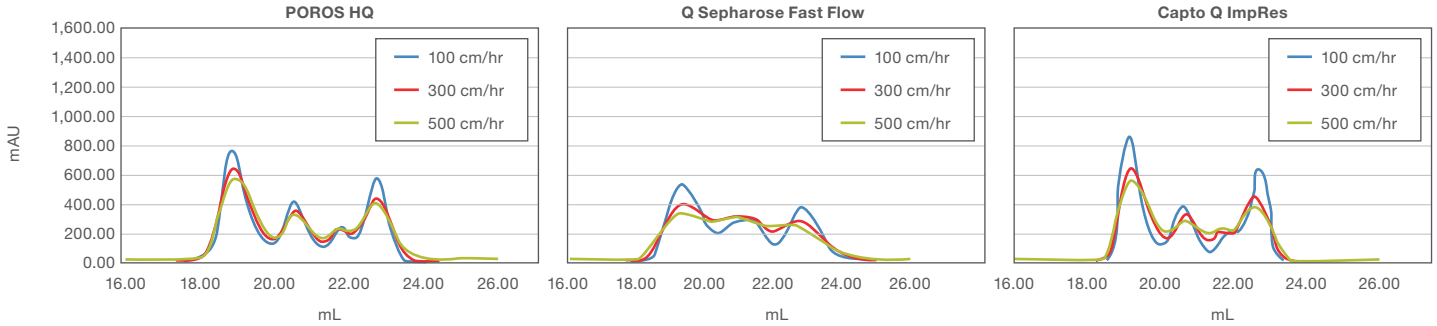


Figure 12. Comparison of resolution vs. flow rate between POROS HQ and other resins. Three columns (0.5 cm ID x 5 cm) packed with 1 mL of either of POROS HQ, GE Healthcare Capto Q ImpRes, or GE Healthcare Q Sepharose™ Fast Flow resin were loaded with a protein mixture of chicken ovalbumin, human holo-transferrin, 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.

Comparison of resolution at different flow rates

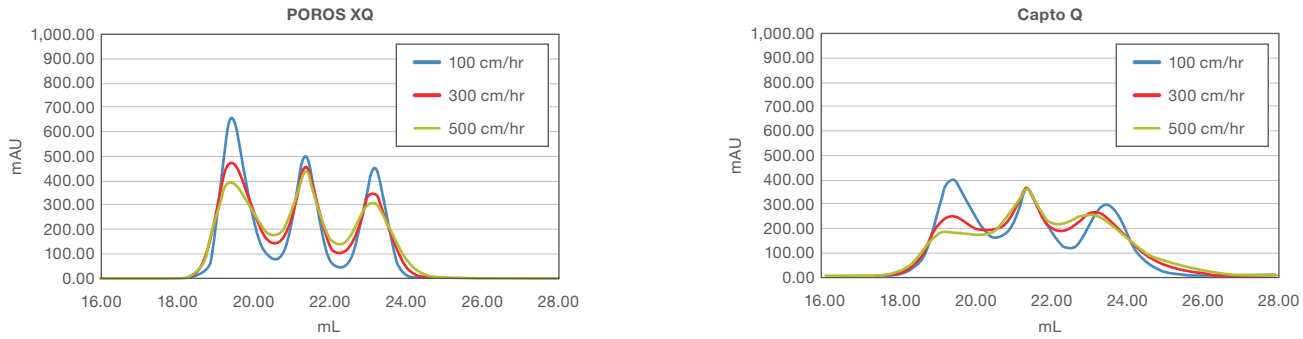


Figure 13. Comparison of resolution vs. flow rate between POROS XQ and GE Healthcare Capto Q resins. Three columns (0.5 cm ID x 5 cm) packed with 1 mL of either of POROS XQ or Capto Q SAX resin were loaded with a protein mixture of chicken ovalbumin, human holo-transferrin, and soybean trypsin inhibitor (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.

Strong cation exchange (SCX) resin and spin columns

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 superior separation capability.

The Pierce Strong Cation Exchange Spin columns are ideal for processing samples of 0.5–20 mL volumes.

Comparison of resolution at different flow rates

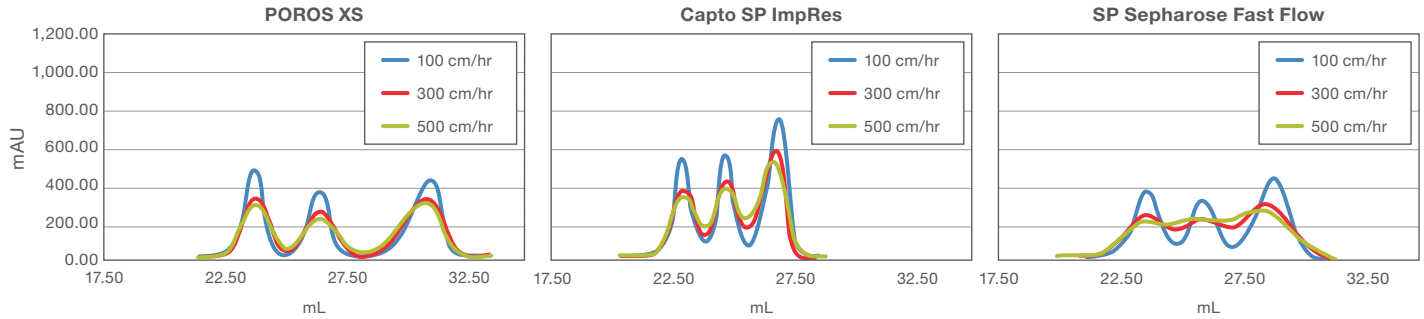


Figure 14. Comparison of resolution vs. flow rate between POROS XS and other SCX resins. Three columns (0.5 cm ID x 5 cm) packed with 1 mL of either POROS XS, GE Healthcare Capto SP ImpRes, or GE Healthcare SP Sepharose Fast Flow resin were loaded with a protein mixture of chymotrypsinogen, cytochrome *c*, and lysozyme (1.5 mg of each protein). A gradient of 0–1 M NaCl was applied 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.

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 ligands enable purification of general immunoglobulins from a crude sample. 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.

Table 12. 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	•				
		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 Magnetic Agarose		•			
		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)	Pierce Mannan Binding Protein Agarose			•		
IgA enrichment	Immobilized jacalin, a D-galactose–binding lectin	Pierce Jacalin Agarose			•		

Comparison of protein yield between suppliers

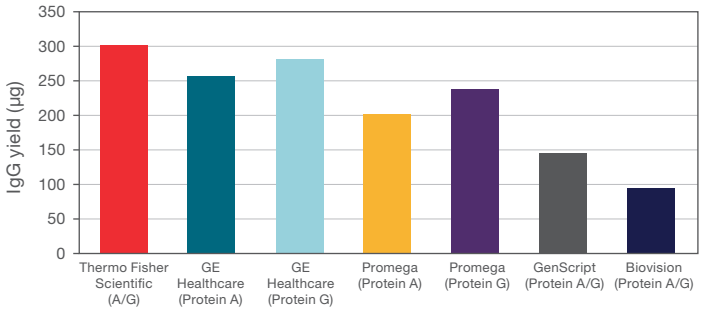


Figure 15. Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads provide higher purification yields than other commercially available magnetic beads. IgG purification was performed with the Pierce Protein A/G Magnetic Agarose Beads, GE Healthcare™ Protein A Mag Sepharose Xtra beads, GE Healthcare Protein G Mag Sepharose Xtra beads, Promega™ Magne™ Protein A beads, Promega Magne Protein G beads, GenScript™ Protein A/G MagBeads, BioVision™ Protein A/G Magnetic Beads, and Pierce Protein A/G Magnetic Beads. Mouse and human sera (50 µL) were diluted with binding buffer according to the manufacturers' protocols and added to magnetic agarose beads. IgG was purified following the manufacturers' protocols. IgG yield was estimated by absorbance of IgG at 280 nm. All purifications were done in duplicate.

Comparison of dynamic binding capacity at different flow rates

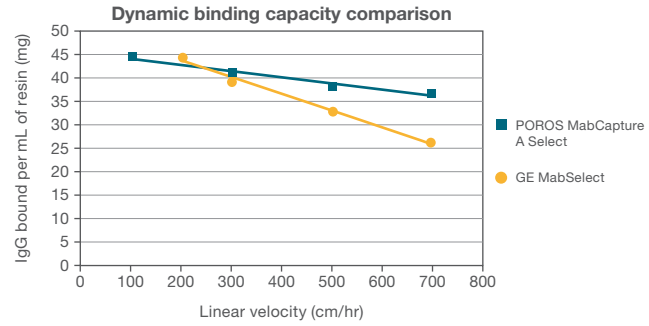


Figure 16. Comparison of dynamic binding capacity vs. flow rate. Two columns (0.46 cm ID x 20 cm) were packed with 1 mL of either Thermo Scientific™ POROS™ MabCapture™ A Select or GE Healthcare MabSelect™ resin and then were challenged with human IgG (5 mg/mL) at flow rates of 700, 500, 300, 200, or 100 cm/hr. The dynamic binding capacity (total protein loaded) was determined at 5% breakthrough.

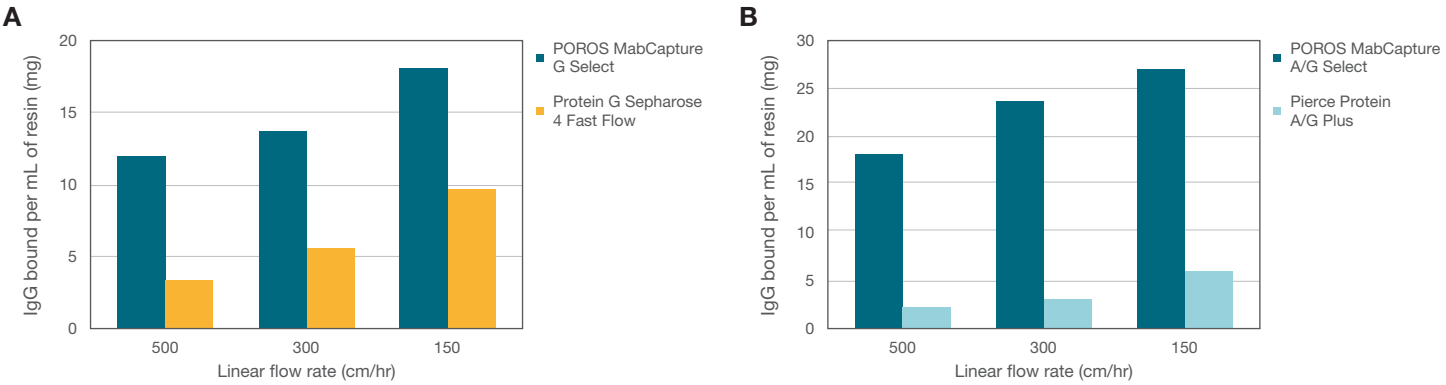


Figure 17. Comparison of dynamic binding capacity vs. flow rate. Each column (0.5 cm ID x 5 cm) was packed with 1 mL of resin and was challenged with human IgG (1 mg/mL) at flow rates of 500, 300, or 100 cm/hr (corresponding to residence times of 0.3, 1, and 2 min, respectively). The dynamic binding capacity (total protein loaded) was determined at 10% breakthrough. **(A)** Comparison between Thermo Scientific™ POROS™ MabCapture™ G Select and GE Healthcare Protein G Sepharose 4 Fast Flow resins. **(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™ resins for the purification of recombinant proteins from cultures such as *E. coli* or *Pichia*. 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™, c-Myc, and HA.

Table 13. 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				•

Protein yield (using densitometry)

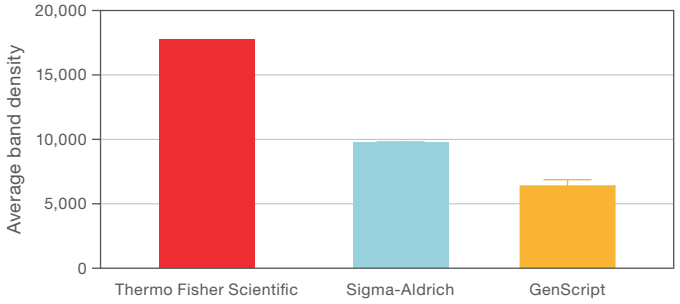


Figure 18. Comparison of DYKDDDDK-tagged SUMO protein purification yields using Thermo Scientific™ Pierce™ Anti-DYKDDDDK Affinity Resin and products from other suppliers. C- and N-terminal DYKDDDDK-tagged SUMO proteins were expressed in *E. coli* and purified using Pierce Anti-DYKDDDDK Affinity Resin, Sigma-Aldrich Anti-FLAG M2 Affinity Gel, and GenScript Anti-DYKDDDDK G1 Affinity Resin. Tagged protein was competitively eluted with Pierce 3x DYKDDDDK Peptide, and the results were analyzed by densitometry using the Invitrogen™ iBright™ Imaging System.

Dynamic binding capacity of anti-DYKDDDDK (anti-FLAG) resin

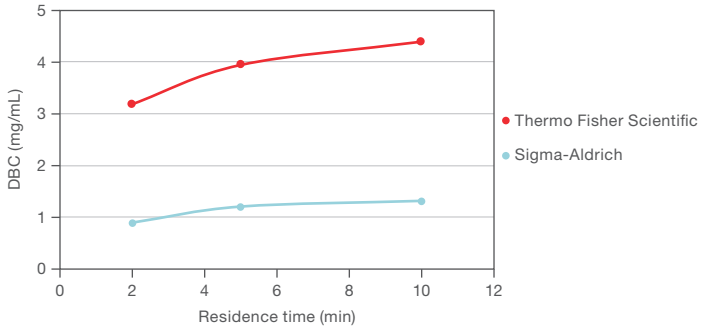


Figure 19. Dynamic binding capacity (DBC) vs. residence time. Pierce Anti-DYKDDDDK Affinity Resin and Sigma Anti-FLAG M2 Affinity Gel were packed into 1 mL columns (0.5 cm ID x 5 cmL) and loaded with purified DYKDDDDK-TurboGFP-His (1 mg/mL) in 100 mM phosphate, 150 mM NaCl, pH 7.2 (PBS), under a variety of residence times (150, 60, and 30 cm/hr) until 10% breakthrough was achieved as measured by A_{280}.

Comparison of protein yield between suppliers

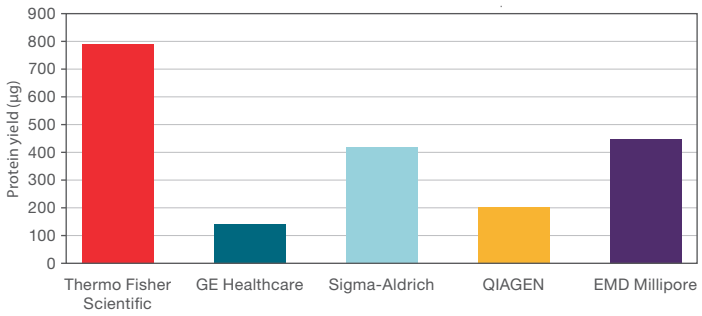


Figure 20. Comparison of protein yield using Thermo Scientific™ Pierce™ Ni-NTA Magnetic Agarose and products from other suppliers. Samples (0.5 mL) of 6xHis-tagged BirA protein were diluted with 0.5 mL binding buffer and purified manually with 25 mL settled beads. Respective suppliers' protocols were followed for their buffer compositions and volumes. Pierce Ni-NTA Magnetic Agarose had the highest yield compared to beads from the other suppliers.

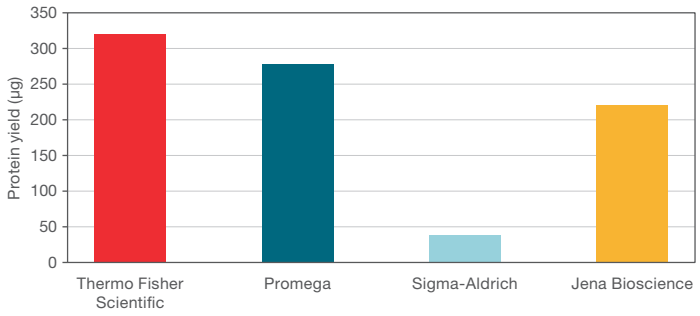


Figure 21. Comparison of protein yield using Thermo Scientific™ Pierce™ Glutathione Magnetic Agarose and products from other suppliers. Samples (0.25 mL) of GST-RalGDS (Ral-guanine nucleotide dissociation stimulator) were diluted with 0.25 mL binding buffer and purified manually with 25 µL settled beads. Respective suppliers' protocols were followed for their buffer compositions and volumes. Pierce Glutathione Magnetic Agarose had the highest yield compared to beads from the other suppliers.

Comparison of protein purity and yield and resin reusability

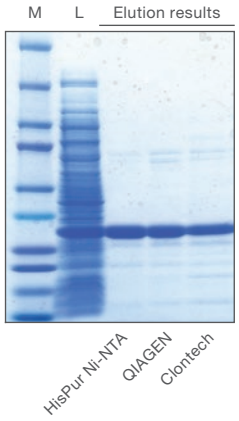
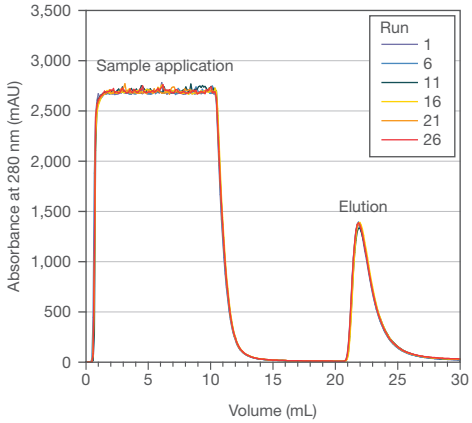


Figure 22. Thermo Scientific™ HisPur™ Ni-NTA Resin performs as well as or better than other suppliers' nickel resins. Bacterial lysate (12 mg total protein) containing overexpressed 6xHis-GFP was applied to HisPur Ni-NTA Resin (Cat. No. 88221) (0.2 mL) and purified by the batch-bind method. The same amount of total protein was applied to QIAGEN and Clontech resins per the manufacturers' instructions. Gel lanes were normalized to equivalent volume. M = molecular weight marker; L = lysate load.



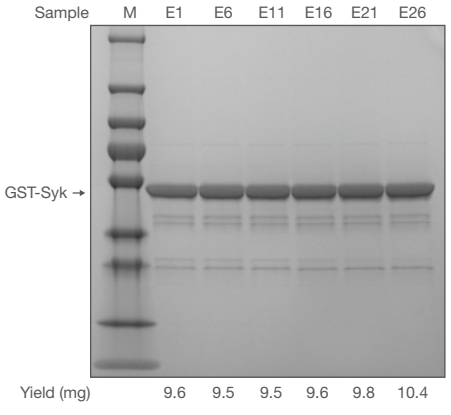


Figure 23. Dependable reusability of Thermo Scientific™ Pierce™ Glutathione Superflow Agarose. Glutathione Superflow Agarose was challenged with multiple rounds of protein purification and column cleaning. An equilibrated 1 mL column (column diameter = 0.5 cm) packed with Glutathione Superflow Agarose 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 with 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 (cycles 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 revealed pure, consistent GST-Syk. M = molecular weight marker.

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. Different biotin-binding ligands are available based on elution conditions or level of purity.

Table 14. Biotin-binding affinity resin selection guide.

Ligand	Specificity	Nonspecific binding	Recommended product	High-throughput screening	Batch
Avidin	Low	High	Pierce Avidin Agarose Resin		•
Monomeric avidin	High	Low	Pierce Monomeric Avidin Resin		•
Streptavidin	Higher	Lower	Pierce Streptavidin Magnetic Beads	•	
			Pierce Streptavidin Agarose Resin		•
			Pierce High Capacity Streptavidin Agarose Resin		•
NeutrAvidin	Highest	Lowest	Pierce NeutrAvidin Agarose Resin		•
			Pierce High Capacity NeutrAvidin Agarose Resin		•

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 24. 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 manufacturers' instructions. Binding capacity was determined using the Pierce BCA Protein Assay Kit.

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. Different reactive chemistries are available to optimize immobilization based on the ligand properties.

Table 15. Activated support selection guide.

Target functional group	Ideal for	Recommended product	High-throughput screening	Batch
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 peptides	CarboxyLink Coupling Resin		•

For more information or to view additional products and pack sizes, 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 a faster, easier, and more efficient way of pulling down the proteins than nonmagnetic methods (Figure 22).

Thermo Fisher Scientific offers 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 more cost-efficient solution per sample
- **Fast and easy**—magnetic beads offer a rapid IP protocol, with no centrifugation or preclearing steps
- **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)

Published papers on immunoprecipitation

All publications

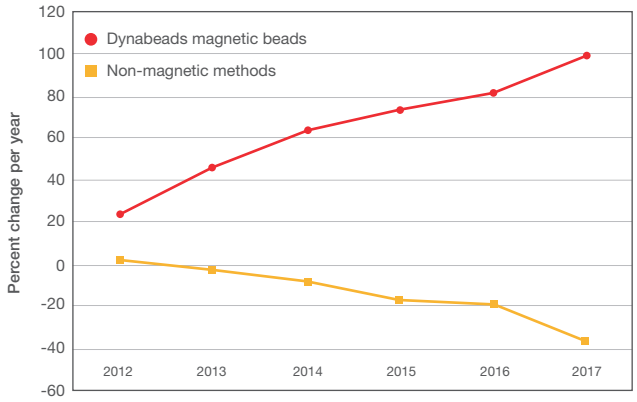


Figure 25. Immunoprecipitation publication growth (Dynabeads magnetic beads compared to nonmagnetic methods). (Source: January 2018 Google Scholar)

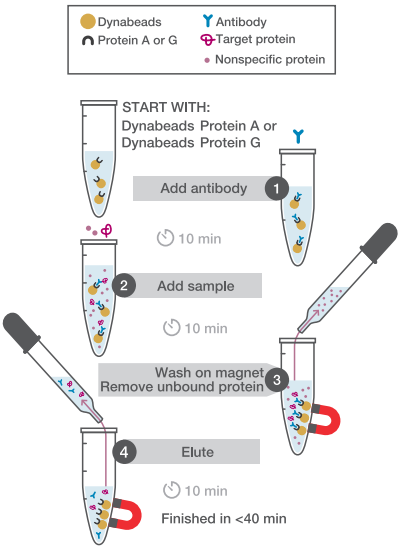


Figure 26. Immunoprecipitation in <40 minutes. Dynabeads magnetic beads precoupled with Protein A or Protein G act as a suspendable solid support that can be fixed by the use of a magnet. This allows for simple and efficient antibody capture, followed by immunoprecipitation of your pure target peptides, proteins, protein complexes, or other antigens.

Table 16. 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: <40 minutes Pierce beads: 130–180 min	• Dynabeads—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: <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: 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: <40 minutes 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 Beads Pierce Anti-DYKDDDDK Magnetic Agarose Pierce HA-Tag Magnetic IP/Co-IP Kit Pierce c-Myc-Tag Magnetic IP/Co-IP Kit

* See more choices in surface-activated Dynabeads products for the binding and capture of additional targets.

Choose these products if you use unconjugated primary antibodies—your choice of antibody-binding products depends on your downstream application, or if you don't 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 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:

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

IP and co-IP strategies using unconjugated primary antibodies

Proteins A, G, A/G, and L have different structures and number of binding sites that influence each ligand's binding affinity to various antibody targets (e.g., antibody subclass or animal species). These ligands result in the effective immunoprecipitation of specific antibodies from a crude sample. Alternate strategies include secondary antibodies to target species specific antibodies, or direct immobilization of target specific antibodies using an activated support. Both convenient kits and stand-alone magnetic beads are available. Protocols for automated, high-throughput IP are available using the Thermo Scientific™ KingFisher™ Flex Purification System.

Protein yield using Dynabeads Protein G and Pierce Protein A/G magnetic beads

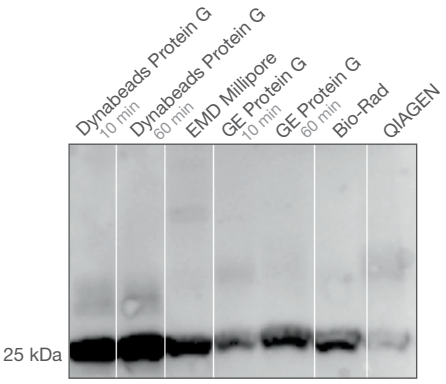


Figure 27. Protein yield results using western blotting. Dynabeads Protein G magnetic beads have the best overall performance in yield, capacity, and nonspecific binding.

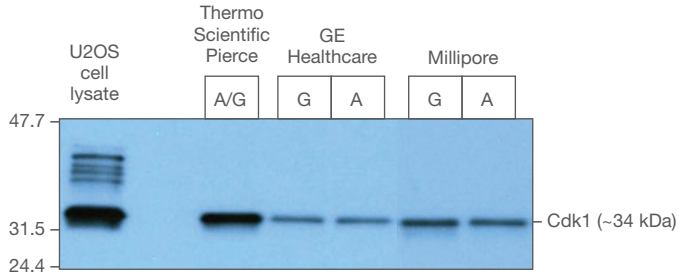


Figure 28. Higher IP yield with Protein A/G beads. U2OS (human osteosarcoma) cells were lysed in IP Lysis/Wash Buffer, and incubated with and without anti-Cdk1 antibody overnight at 4°C. Pierce Protein A/G Magnetic Beads were compared to Mag Sepharose Beads (GE Healthcare) and PureProteome™ (EMD Millipore) Protein A and Protein G products. The beads were washed multiple times using the 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.

Nonspecific binding results using Dynabeads Protein G

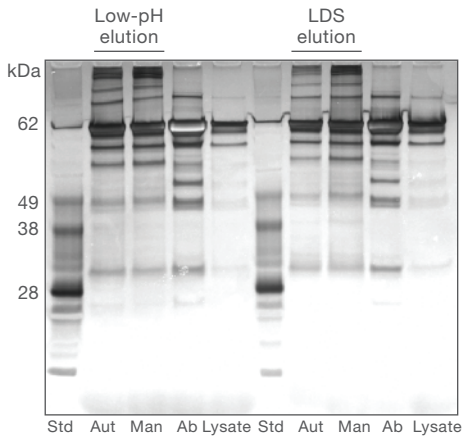


Figure 29. Low nonspecific binding with manual and automated immunoprecipitation. Immunoprecipitation from HeLa cell lysate (Lysate) with Dynabeads Protein G bound to an irrelevant antibody (Ab) using either manual (Man) or automated protocol (Aut) on the KingFisher Flex instrument. Mild or denaturing elution conditions were used followed by silver staining (Invitrogen™ SilverQuest™ Silver Staining Kit) of gels (Invitrogen™ Bolt™ 4–12% Bis-Tris Plus). The automated protocol had just as low nonspecific binding as the manual protocol using both elution conditions.

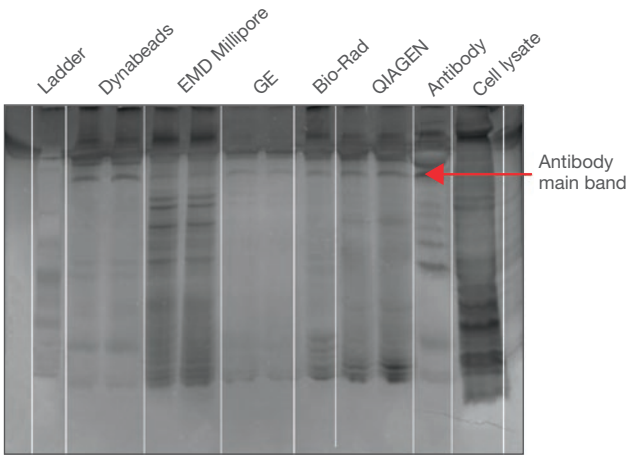


Figure 30. Nonspecific binding results using silver staining. Dynabeads Protein G magnetic beads show very little nonspecific binding, and provide the best signal-to-noise ratio when compared to other suppliers.

IP effectiveness using the direct immoblilization of antibodies

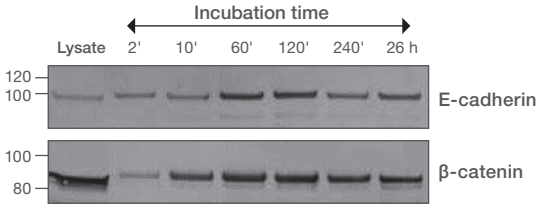


Figure 31. IP/co-IP results using the Invitrogen™ Dynabeads™ Co-Immunoprecipitation Kit. This kit was used to co-precipitate E-cadherin and β-catenin from a cell lysate. Antibody against E-cadherin was covalently bound to the epoxy-based Dynabeads magnetic beads to achieve high yield and ultralow nonspecific binding without the need for crosslinking. Incubation times as low as 10 minutes were sufficient to achieve good yields of both proteins. The buffers in the kits were optimized in collaboration with members of the Routs lab at Rockefeller University, NY.

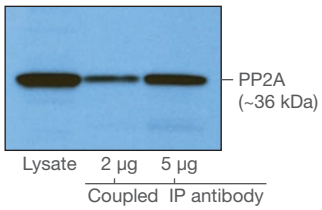


Figure 32. Excellent immunoprecipitation results with the Thermo Scientific™ Pierce™ Direct Magnetic IP/Co-IP Kit. Following the kit procedure, anti-PP2A antibody (2 µg and 5 µg) was coupled to 25 µL of Thermo Scientific™ Pierce™ NHS-Activated Magnetic Beads and then used to immunoprecipitate PP2A from 0.5 mg aliquots of an A431 (human epidermoid carcinoma) cell lysate. The eluates were resolved by SDS-PAGE and analyzed by western blot for PP2A. The Pierce NHS-Activated Magnetic Beads effectively immunoprecipitated PP2A using as little as 2 µg of antibody.

IP, co-IP, and pull-down strategies using biotinylated antibodies

Streptavidin-based magnetic beads exploit the strong association between avidin and biotin molecules, a nearly irreversible bond. Streptavidin magnetic beads are ideal for the immunoprecipitation of antigens using biotinylated antibodies from a wide variety of sources. The effective co-IP of interaction complexes can be achieved using biotinylated antibodies, as well as for the capture of interacting proteins in pull-down assays using biotinylated "bait" proteins. Both convenient kits and stand-alone magnetic beads are available. Protocols for automated, high-throughput IP are available using the KingFisher Flex Purification System.

Benching binding capacity and elution efficiency using Pierce Streptavidin Magnetic Beads and the IP-MS kit

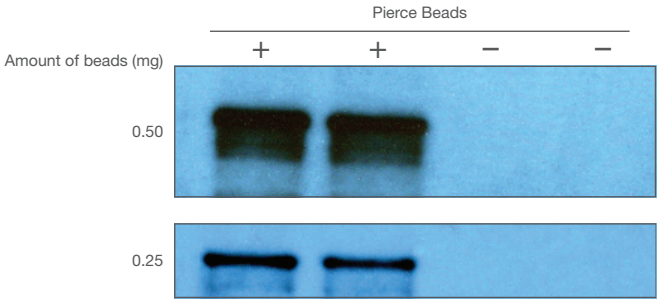


Figure 33. High-capacity immunoprecipitation results with Thermo Scientific™ Pierce™ Streptavidin Magnetic Beads. MOPC cell lysate (0.75 mg per sample) was incubated overnight at 4°C with and without 10 µg biotinylated Grp94 antibody. Pierce Streptavidin Magnetic Beads (Cat. No. 88817) were added to a 96 deep well plate (0.5 mg or 0.25 mg per well). Using the KingFisher 96 Instrument, the beads were washed with Tris-buffered saline containing 0.1% Tween 20, incubated 1 hour with the antigen and antibody mixture sample, washed three times and then eluted for 10 minutes at 96°C with SDS-PAGE reducing sample buffer. Eluates were resolved by SDS-PAGE and analyzed by western blot with anti-Grp94 antibody.

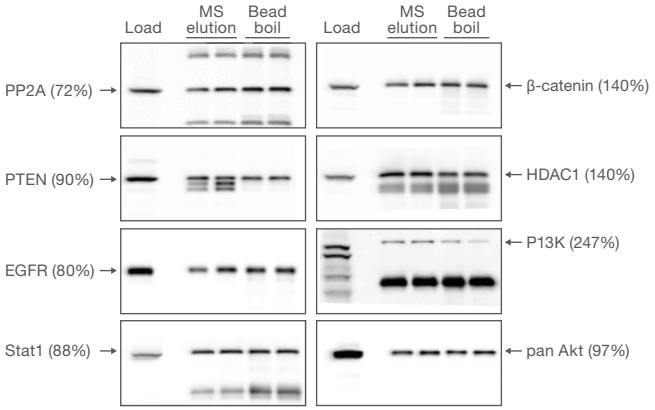


Figure 34. The Thermo Scientific™ Pierce™ MS-Compatible Magnetic IP Kit (streptavidin) allows for effective target capture and elution. Percentages beneath target indicate elution efficiency compared to bead boil. The elutions were analyzed by western blot. Antibodies were labeled with the Thermo Scientific™ Pierce™ Antibody Biotinylation Kit for IP and used with the kit to immunoprecipitate target proteins from cell lysates.

Table 17. List of co-immunoprecipitated proteins. The Pierce MS-Compatible Magnetic IP Kits showed effective co-IP of interacting proteins for CTNNB1, EGFR, PI3KCA, CBP, NOTCH1, AKT, AKT1, SMAD4, and/or ARAF targets. These are known protein interactions reported in previous studies. **Panel A:** Streptavidin Kit. **Panel B:** Protein A/G Kit.

Panel A

IP target	Co-IP proteins
CTNNB1	CTNNA1, CDH2, CDH11, APC, ARVCF, PKP4
EGFR	PRKDC, PFKP, SL C3A2, RPN1
PI3KCA	PIK3R2, PIK3R1
CBP	PSMC5, ACTA2, DDX5
AKT	VIM, HSPA8, TUBA1A
SMAD4	EEF1A1, SQSTM1

Panel B

IP target	Co-IP proteins
CTNNB1	CTNNA1, CDH11, CDH2, CTNND1
EGFR	TUBB, TUBA1A, HSPA1A
PI3KCA	PIK3R2, PIK3R1
NOTCH1	PTBP1, C14orf166
AKT1	AKT2, ACTB
ARAF	YWHAG, STK25

IP, co-IP, and pull-down strategies using recombinant tags

Recombinant tags such as 6xHis, FLAG, GST, c-Myc, and HA enable the IP, co-IP, or pull-down of recombinant proteins or protein complexes. Pull-down is achieved using immobilized Ni-NTA, cobalt, or glutathione. IP and co-IP can be performed using immobilized anti-FLAG, anti–c-Myc, or anti-HA antibodies. Both convenient kits and stand-alone magnetic beads are available. Protocols for automated, high-throughput IP are available using the KingFisher Flex Purification System.

IP and pull-down results using Dynabeads and Pierce magnetic beads or magnetic agarose

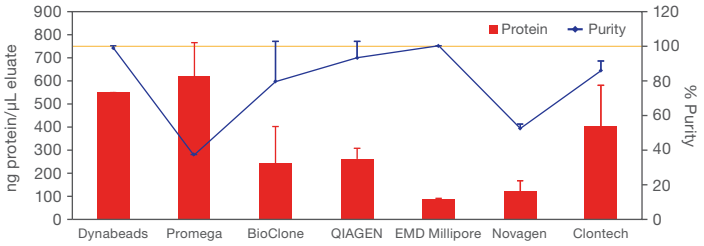


Figure 35. High yield and purity of polyhistidine-tagged proteins in 20 minutes. Invitrogen™ Dynabeads™ His-Tag Isolation and Pulldown beads were used to purify GFP-labeled, polyhistidine-tagged proteins in an *E. coli* lysate and compared to similar products from different suppliers. GFP fluorescence was used to detect the protein concentration (yield), and an Agilent Bioanalyzer™ instrument was used to measure the purity. The Dynabeads kit provides the best combination of reproducible high yield with excellent purity, in only 20 minutes.

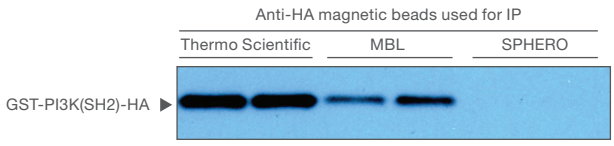


Figure 36. Better immunoprecipitation results with Thermo Scientific™ Pierce™ Anti-HA Magnetic Beads. Using a KingFisher Flex Purification System with 96 deep-well plates, 25 µL of Pierce Anti-HA Magnetic Beads, Anti-HA-tag Magnetic Beads (MBL International Corp.), and SPHERO™ Rabbit Anti-HA Magnetic Beads (Spherotech Inc.) were used to immunoprecipitate GST-PI3K(SH2)-HA from 50 µg of *E. coli* lysate in duplicate. Captured protein was eluted with 0.1 M glycine, pH 2.0, and then resolved by SDS-PAGE and analyzed by western blot for the HA-tagged protein.

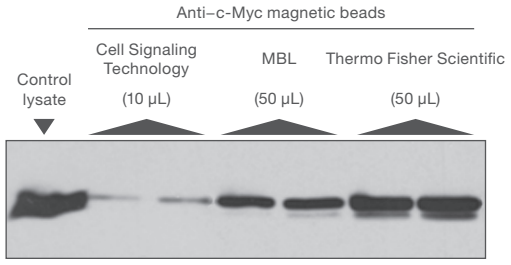


Figure 37. Better immunoprecipitation results with Thermo Scientific™ Pierce™ Anti–c-Myc Magnetic Beads. Green *Renilla* luciferase c-Myc fusion protein was expressed in 293T cells. For IP, identical aliquots of the cell lysate were incubated in duplicate for 1 hour at room temperature with anti–c-Myc magnetic beads from each manufacturer. For all conditions, IP products were eluted identically using low-pH buffer. Eluted fractions (25 µL each) were separated by 12% SDS-PAGE, transferred to PVDF membranes, and detected via anti–c-Myc antibody, goat anti-mouse secondary antibody, and chemiluminescent substrate.

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

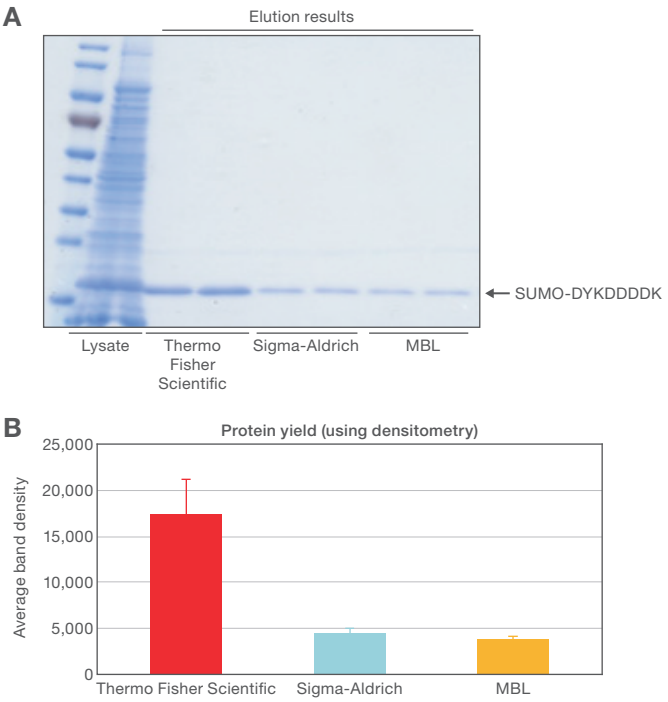


Figure 38. Comparison of DYKDDDDK-tagged SUMO protein yield and background using Pierce anti-DYKDDDDK resin and products from other suppliers. C- and N-terminal DYKDDDDK-tagged SUMO proteins were expressed in *E. coli* and purified using Pierce Anti-DYKDDDDK Magnetic Agarose, Sigma-Aldrich Anti-FLAG™ M2 Magnetic Beads, and MBL Anti-DDDDK-tag mAb-Magnetic Agarose. Tagged protein was competitively eluted with Pierce 3x DYKDDDDK Peptide, and the results were analyzed by SDS-PAGE **(A)** and densitometry using the Invitrogen™ iBright™ Imaging System **(B)**. Comparison between the starting lysate and elution fractions shows effective immunoprecipitation and elution of DYKDDDDK-tagged protein, with minimal background from the Pierce magnetic agarose compared to the other suppliers' products.

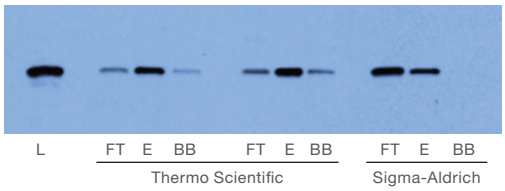


Figure 39. Comparison of protein purification results using Pierce Anti-DYKDDDDK Affinity Resin and another supplier's product. C-terminal DYKDDDDK-tagged GFP protein was expressed using the Thermo Scientific™ 1-Step Human High-Yield Maxi *In Vitro* Translation (IVT) Kit and immunoprecipitated using Pierce Anti-DYKDDDDK Affinity Resin or Sigma Anti-FLAG M2 Affinity Gel. Tagged proteins were competitively eluted with Pierce 3x DYKDDDDK Peptide and analyzed by western blot. Comparison of the starting lysate (L), flow-through (FT), elutions (E), and bead-boiled samples (BB) shows effective capture and elution of DYKDDDDK-tagged proteins.

Magnetic stands

Multiple formats for low- to high-throughput sample processing

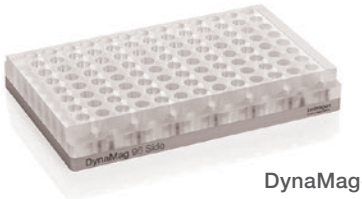
Highlights:

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

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

The Invitrogen™ DynaMag™-2 Magnet (shown above) holds up to 16 standard 1.5–2 mL tubes and is optimal for working volumes of 10 to 2,000 µL. The top rack can be quickly removed from the magnet in the base, ready for vortexing, rotation, or manual sample shaking.

Plate-based magnetic stands, such as the Invitrogen™ DynaMag™-96 series magnets, are ideal for manual and automated work, with a footprint that is the same as that of a 96-well plate. The recommended working volume is 5–200 µL.



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

Learn more at **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**		
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**		
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	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
Pierce Protease Inhibitor XL Capsules, EDTA-free	10 capsules	A37989
Halt Phosphatase Inhibitor Cocktail (100X)	1 mL	78420
Pierce Phosphatase Inhibitor Mini Tablets	20 tablets	A32957
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	A32959
Pierce Protease and Phosphatase Inhibitor Mini Tablets, EDTA-free	30 tablets	A32961
To view additional pack sizes and products, go to **thermofisher.com/inhibitorcocktails**		

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//pk	88513
Pierce Protein Concentrator PES, 10K MWCO, 2–6 mL	24//pk	88517
Pierce Protein Concentrator PES, 10K MWCO, 5–20 mL	24//pk	88528
Pierce Protein Concentrator PES, 10K MWCO, 20–100 mL	24//pk	88535
To view additional pack sizes and MWCOs, go to **thermofisher.com/concentrators**		

Ordering information

Product	Quantity	Cat. No.
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		
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
Pierce Protein A/G Magnetic Agarose Beads	1 mL	78609
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 Kit	Kit	45214
Recombinant protein purification resins and magnetic beads		
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	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

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Product	Quantity	Cat. No.
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	2 mL	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

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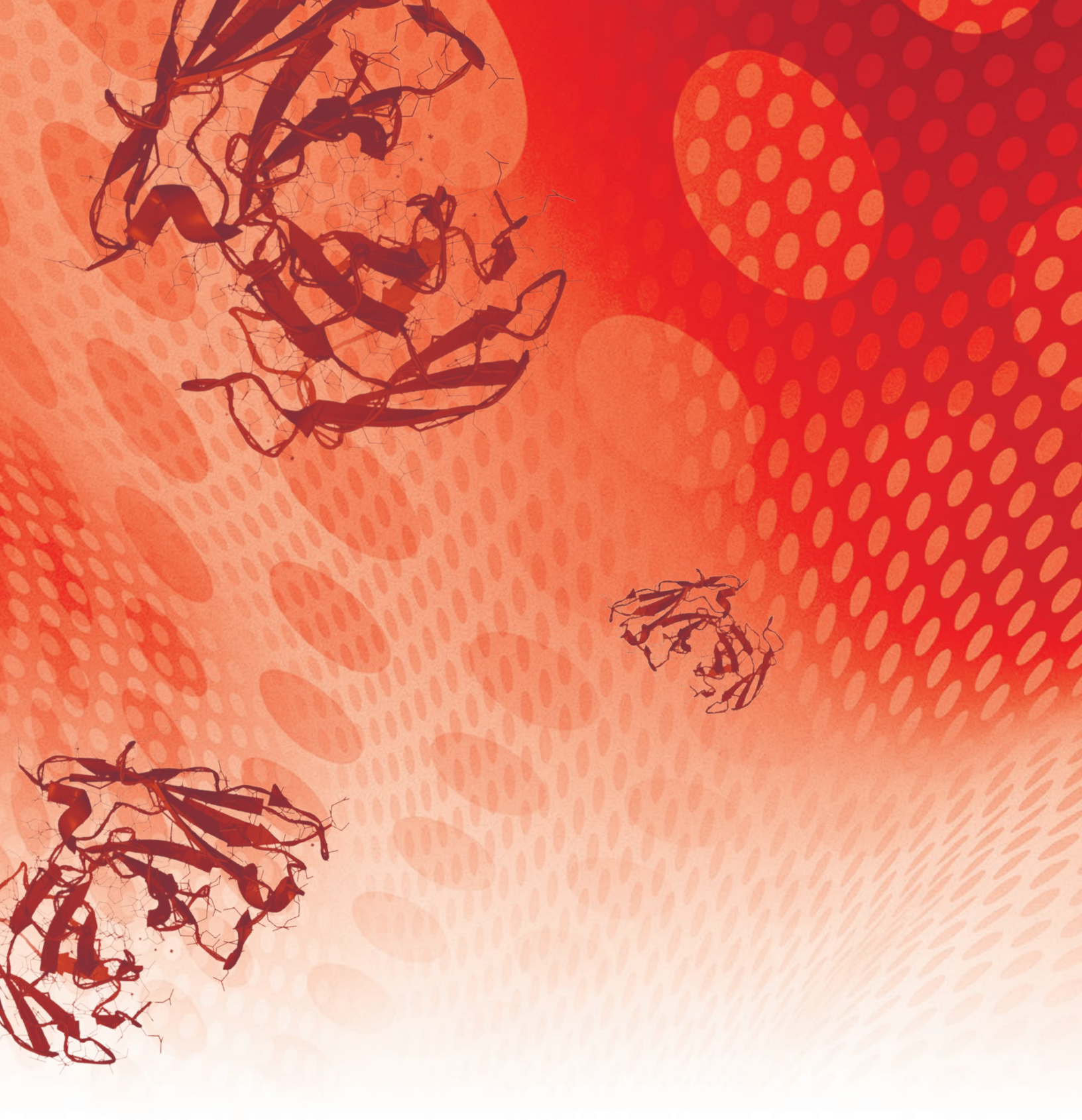

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