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Protein clean-up technical handbook

Dialysis • Desalting • Detergent removal •
Concentration • Endotoxin removal

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Contents

Overview of protein clean-up methods

The first step in protein analysis is cellular extraction. Following lysis, and depending on the next step in the workflow, the protein extract may require further clean-up or enrichment during downstream processing, using techniques such as dialysis, desalting, concentration, or contaminant-specific removal.

Historically, mechanical disruption has been used to lyse cells and tissues; however, detergent-based solutions have more recently been developed to efficiently lyse cells and enable the separation of subcellular structures without requiring physical disruption. Many detergents, salts and other molecules used in or generated during protein extraction or purification may have adverse effects on protein function or stability, or interfere with downstream applications; therefore it may be necessary to remove or reduce these contaminants using one or more of the following methods.

Dialysis

Dialysis is a separation method that utilizes selective diffusion through a semi-permeable membrane to remove small contaminants or exchange buffers from proteins in solution. Proteins that are larger than the membrane pore size are retained on one side of the membrane while small molecular weight contaminants diffuse freely through the membrane and approach an equilibrium concentration. Flat dialysis tubing composed of cellulose acetate or regenerated cellulose was introduced in the 1950s. This format requires preparation and is cumbersome and difficult to handle. Thermo Scientific™ dialysis products are essentially ready to use and are designed to eliminate potential sample leakage and maximize ease of use for specific applications.

Desalting

Size exclusion chromatography (also known as gel filtration or molecular sieve chromatography) can be effectively utilized for protein desalting (removal of salt from a sample). A resin is selected with pores that are large enough to trap small contaminants (e.g., salts), but too small for the protein of interest to enter. The larger, faster proteins separate from the slower, smaller molecules and can be collected first. The Thermo Scientific™ Zeba™ desalting products contain a unique resin that enables exceptional desalting and protein recovery, and are available in convenient spin columns and plate formats that allow samples to be processed in minutes.

Detergent removal

Detergent removal has traditionally utilized a variety of methods including dialysis, ion exchange chromatography, sucrose gradients, or acid or acetone precipitation. However, all these methods can be labor- and/or time-intensive, or detergent-specific. The proprietary Thermo Scientific™ Detergent Removal resins enable the efficient, rapid and effective extraction of a wide variety of detergents (ionic, nonionic and zwitterionic) that are commonly used in protein extraction, purification and biological assays.

Concentration

Protein concentration utilizes a semi-permeable membrane to separate macromolecules from low molecular weight compounds. Unlike dialysis, which relies on passive diffusion, concentration is achieved by forcing solutions through the membrane by centrifugation. Solvents and small molecular weight molecules pass through the membrane pores as the protein solution is forced against the membrane barrier in a centrifuge tube, concentrating the macromolecules (e.g., proteins) in the remaining solution (retentate). For buffer exchange (diafiltration), the concentrated solution is diluted and concentrated multiple times until the desired state is achieved. The easy-to-use Thermo Scientific™ Pierce™ Protein Concentrators contain a high performance PES (polyethersulfone) membrane that enables fast processing and excellent protein recovery for samples.

Thermo Fisher Scientific offers a variety of specialty devices and resins that simply and efficiently desalt, exchange buffer, and remove detergents from samples. In addition, if the protein sample is too dilute for further processing or analysis, the sample can be concentrated quickly using centrifugal concentrators (Table 1).

Endotoxin removal

Endotoxin contamination is a common problem with recombinant proteins purified from gram-negative bacteria such as *E. coli*. Traditional endotoxin removal methods include anion-exchange chromatography, ultrafiltration, membrane-based chromatography, and Polymixin B affinity ligand. These methods are limited by specificity, capacity or reusability. Thermo Scientific™ Pierce™ High Capacity Endotoxin Removal Resin selectively binds and removes endotoxins from protein, peptide and antibody samples using a modified ε-poly-L-lysine [poly(ε-lysine)] affinity ligand.

Table 1. Protein clean-up selection guide

	Dialysis devices, cassettes, 96-well plates and tubing	Desalting spin columns, 96-well spin plates and cartridges	Detergent removal spin columns and 96-well spin plates	Concentrators
				
Sample volume processing range	10 µL–250 mL	2 µL–4 mL	25 µL–1 mL	100 µL–100 mL
Sample processing time (volume dependent)	2–24 hrs	5–10 min	15–20 min	5–30 min*
Key applications	Buffer exchange, desalting, virus purification	Desalting, buffer exchange, removal of unincorporated label	Removal of anionic, nonionic and zwitterionic detergents	Sample concentration, desalting, buffer exchange
Recommended sample type	Purified protein	Lysate or purified protein	Purified protein or peptide mixture	Lysate or purified protein
Compatible with viscous samples?	Yes	No	No	No
Sample diluted during processing?	Possible	No	No	No
Gamma-irradiated options available?	Yes	No	No	No

* For 10K MWCO (device dependent—times may vary for other MWCOs)

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

Protein dialysis using regenerated cellulose membranes

Overview

Dialysis is a classic solution-based separation technique that facilitates the removal of small, unwanted compounds from macromolecules by selective diffusion. In a typical dialysis application, a sample and a buffer solution are placed on opposite sides of a semi-permeable membrane. Sample molecules that are larger than the membrane pores are retained on the sample side of the membrane, but small molecules diffuse freely through the membrane and approach an equilibrium concentration with the entire buffer volume (Figure 1).

Through this process, the concentration of small contaminants in the sample can be decreased to acceptable or negligible levels if the external buffer volume is large (typically 30–500 times the sample volume). Alternatively, desired components in the external buffer solution can be slowly brought into the sample. Dialysis is used for a wide variety of applications including simple salt removal and buffer exchange, removal of labeling reagents, drug binding studies, cell growth and feeding, and virus purification.

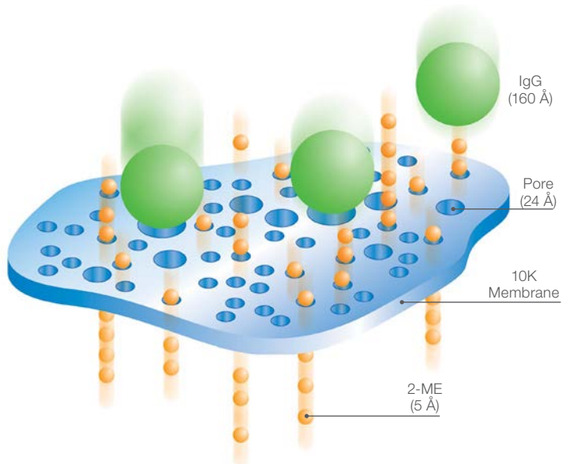


Figure 1. How dialysis membranes work. A dialysis membrane is a semi-permeable film (usually a sheet of regenerated cellulose) containing various sized pores. Molecules larger than the pores cannot pass through the membrane, but small molecules can do so freely. In this manner, dialysis may be used to perform purification or buffer exchange for samples containing macromolecules.

Factors that influence dialysis

Dialysis is used for separating molecules with significantly different molecular weights (typically a 10- to >50-fold size difference). The diffusion properties of a membrane are determined by the average or maximum size of its pores, the number of pores and the thickness of the membrane. Membrane selection is based on the molecular weight cutoff (MWCO), which is defined as the average molecular weight of a molecule that can no longer diffuse across the membrane, such that it is retained at >90%, as it no longer fits into the pores. Understanding the significance of a membrane's MWCO and how it behaves enables selection of the best membrane for a particular dialysis application. Molecular weight cutoff is not a "defined" value, as diffusion of molecules near but below the MWCO will also be significantly slowed. Molecules with a molecular weight that is less than 1/10 of the MWCO rating of the membrane will diffuse most rapidly and reliably across the membrane (see data, page 6). A membrane with the proper MWCO will prevent loss of proteins of interest and ensure adequate removal of contaminants.

Although the membrane and its properties are the primary factors that affect dialysis rate, a variety of other factors can also influence dialysis. These include temperature; the geometry, concentration, interactions and hydrophobicity of the molecules; as well as the volume, agitation and frequency exchange of the external buffer. The rate of dialysis is also directly proportional to the surface area of the membrane in relationship to the volume of the sample and the average distance of the sample from the membrane. The more that a sample can be spread over a membrane surface, the faster dialysis will proceed because all molecules in the sample will be closer to the membrane, and a higher proportion of them will be in direct contact with the membrane at any instant.

Optimized products for easier, efficient dialysis

Using dialysis to separate complex mixtures of biomacromolecules was established in the 1950s. Many of the dialysis theories established at that time are the cornerstones for contemporary dialysis products. However, significant improvements have been made to the dialysis tools of yesterday, including faster preparation time, ease of use and reliability. Thermo Scientific dialysis products are essentially ready to use and are designed to eliminate potential sample leakage and maximize ease of use for specific applications. The high performance Thermo Scientific™ Slide-A-Lyzer™ cassettes and flasks are designed to maximize surface-area-to-volume ratios (within practical limits) for different sample volumes. Our broad product portfolio covers a variety of formats and membrane molecular weight cutoffs (MWCOs) to accommodate a wide range of sample volumes and workflows.

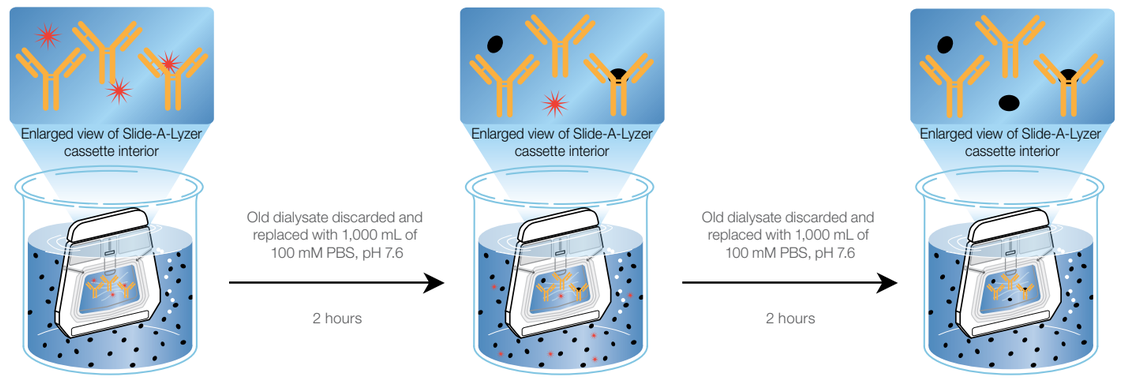


Figure 2. How the Thermo Scientific Slide-A-Lyzer cassette works.

Membrane performance data

Protein recovery by MWCO

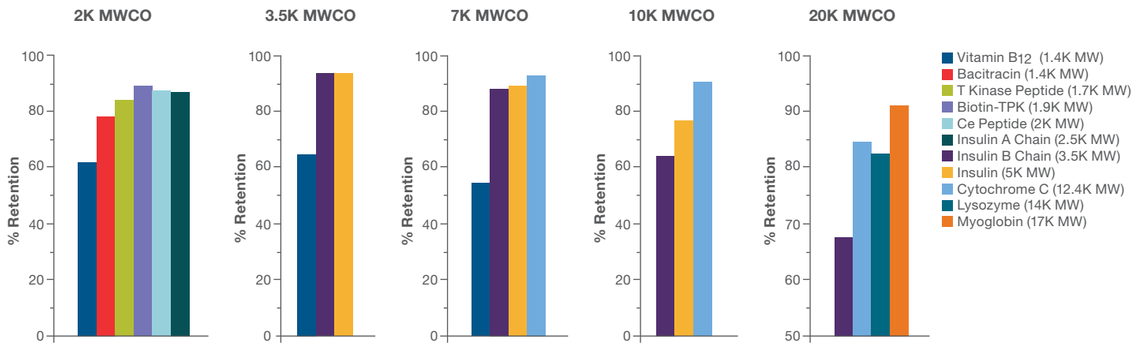


Figure 3. Sample retention by the 2K, 3.5K, 7K, 10K and 20K MWCO Thermo Scientific Slide-A-Lyzer Dialysis Cassette membrane. Individual proteins (1 mg/mL) in either saline or 0.2 M carbonate-bicarbonate buffer, pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of retentate was estimated using either the Thermo Scientific™ Pierce™ BCA Protein Assay or absorption at 360 nm (for vitamin B12).

Protein recovery for specific devices

Table 1. High protein recovery is obtained using the 2 mL Thermo Scientific™ Slide-A-Lyzer™ MINI Dialysis Device.†

Membrane MWCO (K)	Protein/Peptide	Recovery (%)
3.5	Insulin Chain B (3.5 kDa)	90.13
10	Cytochrome C (12.4 kDa)	94.44
20	Myoglobin (17 kDa)	95

† Insulin chain B, cytochrome C and myoglobin (0.25 mg/mL) in either 50 mM sodium phosphate, 75 mM NaCl at pH 7.2 or 0.2 M carbonate-bicarbonate buffer at pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of protein in the retentate was determined using the Thermo Scientific™ Pierce™ BCA Protein Assay (Cat. No. 23225).

Table 2. Quantitative sample recovery using a Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassette vs. conventional tubing. Three sample volume batches of water (0.5 mL, 1.7 mL and 3.0 mL) were loaded and recovered per the respective manufacturer's instructions in a Slide-A-Lyzer Dialysis Cassette or conventional dialysis tubing to determine the volumes of recovery. Water volume recovered was determined gravimetrically.

Sample volume loaded	Slide-A-Lyzer Dialysis Cassette % volume recovery	Traditional dialysis tubing % volume recovery
3.0 mL	99.47	92.32
1.7 mL	99.30	93.12
0.5 mL	98.76	87.51

Membrane specifications by MWCO

Specifications	Slide-A-Lyzer Membrane MWCO					SnakeSkin Tubing
	2K	3.5K	7K	10K	20K	3.5K, 7K and 10K
Membrane composition	all membranes composed of regenerated cellulose					
Required hydration	2 minutes	30 seconds	30 seconds for low-volume samples	30 seconds	2 minutes	none
Glycerol content	none	trace	13%	21%	none	varies with MWCO
Sulfur content	0.169%	0.1–0.15%	0.1–0.15%	0.05%	0.04%	0.1–0.15%
Heavy metals content	trace	trace	trace	trace	trace	trace

Dialysis rates for various formats

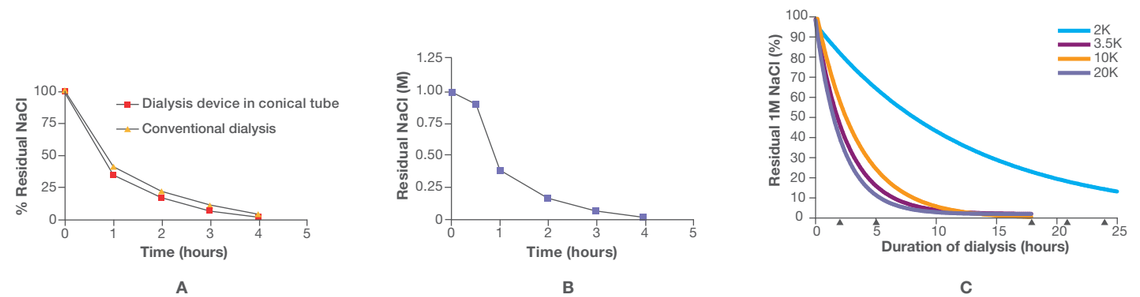


Figure 4. The rate of removal of NaCl using various dialysis products. NaCl removal from samples was determined by measuring the conductivity of the retentate at the indicated times. **Panel A:** Slide-A-Lyzer MINI Dialysis Device (10K MWCO, 2 mL) versus conventional dialysis. Bovine serum albumin (BSA) 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 Thermo Scientific™ Pierce™ 96-well Microdialysis Plate against 1.8 mL of water in a 96-well deep-well plate 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 1M NaCl were dialyzed at room temperature using Thermo Scientific™ Slide-A-Lyzer™ Dialysis Flasks in 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).

Membrane chemical compatibility guide

Note: The following ratings refer to chemical compatibility with the regenerated cellulose dialysis membrane. The plastic cassette frame and silicone-like gasket may leach, dissolve, deform, or otherwise fail in certain strong acids and bases, alcohols, aromatic and chlorinated hydrocarbons, and other chemicals (see asterisks in table) that are listed as being compatible with the dialysis membrane. Test solvents with a cassette before attempting to dialyze valuable samples.

Acetic acid, 25%	G	Ethyl acetate	G*	Nitric acid, <5%	G
Acetone	G*	Ethylene glycol	G	Nitric acid, >25%	N
Ammonium hydroxide, 1 N	F	Formaldehyde solution, 30%	G	Perchloric acid, 25%	N
Ammonium hydroxide, 25%	F	Formic acid, 25%	G*	Phosphoric acid, 25%	F
Ammonium sulfate, 1 M	G	Formic acid, 100%	G*	Potassium hydroxide, 1 N	N
Amyl acetate	G*	Hexane	G*	Propylene glycol	G
Benzene	G*	Hydrochloric acid, <5%	G	Sodium hydroxide, 0.1 N	G
Benzyl alcohol	G*	Hydrochloric acid, >25%	N	Sodium hydroxide, 1 N	F
Butanol	G*	Hydrofluoric acid, 25%	F	Sulfuric acid, <5%	G
Butyl acetate	G*	Hydrogen peroxide, 30%	G	Sulfuric acid, >25%	N
Carbon tetrachloride	G*	Iodine solutions	N*	Tetrahydrofuran	G
Chloroform	G*	Isopropyl alcohol	G	Toluene	G*
Dimethylformamide	F*	Methanol, <50%	G*	Trichloroacetic acid, <10%	F
Dioxane	F	Methyl acetate	G*	Trichloroacetic acid, >25%	N
Ethanol, 70%	G	Methyl ethyl ketone	G*	Trichloroethylene	G*
Ethanol, 95%	G	Methylene chloride	G*	Xylene	G*

Legend: G = Good resistance; F = Fair resistance (pore swelling may occur); N = Not Recommended

* Chemicals known to adversely affect the plastic cassette frame; brief or dilute exposure may be compatible.

Selecting the right format for sample processing

Thermo Scientific dialysis products have evolved over the last decade to meet a variety of customer needs. Our broad portfolio of high performance tools for dialysis, buffer exchange, and sample clean up enables easy handling, enhanced sample protection, and excellent recovery for small to larger volumes. Available in a variety of formats, it's easy to find the best dialysis product for your application.

Table 3. Dialysis products selection guide

	Pierce 96-well Microdialysis Plates	Slide-A-Lyzer MINI Dialysis Devices	Slide-A-Lyzer Dialysis Cassettes (original)	Slide-A-Lyzer G2 Dialysis Cassettes	Slide-A-Lyzer Dialysis Flasks
					
External float required?	No	No	Yes	No	Optional
Method of sample addition/removal	Pipette	Pipette	Syringe	Pipette or syringe	Pipette
Volume range	10–100 µL	10 µL–2 mL	0.1–30 mL	0.25–70 mL	150–250 mL
# of devices with different volume capacities	1	3	4	5	1
Molecular weight cutoffs (MWCOs)	3.5K, 10K	2K (0.1 mL), 3.5K, 10K, 20K	2K, 3.5K, 7K (0.1, 0.5 mL), 10K, 20K	2K, 3.5K, 7K (0.1, 0.5 mL), 10K, 20K	2K, 3.5K, 10K, 20K
Automation compatible?	Yes	No	No	No	No
Integrated buffer reservoir?	Yes	Yes, except 0.1 mL size	No	No	No
Gamma-irradiated options?	No	No	Yes, 10K only	Yes, 10K only	No
Molecular weight cutoff (MWCO) color coded?	No	Yes	Yes	Yes	Yes

Thermo Scientific Pierce 96-well Microdialysis Plate

High-throughput dialysis in a 96-well plate

The Thermo Scientific Pierce 96-well Microdialysis Plate is an automation-compatible system for simultaneously dialyzing between 1 and 96 samples with volumes from 10 µL to 100 µL. The dialysis inserts are provided in strips of eight preloaded in a 96-well deep-well plate, but can be separated easily for use as individual dialysis devices in a standard microcentrifuge tube.

Each microdialysis device has two regenerated cellulose membranes separated by <2 mm. This combination of short diffusion distance and large surface area allows for rapid dialysis. In addition, the small distance between the membranes allows highly efficient sample recovery using standard laboratory pipettes. The dialysis chambers come in strips of eight units that can be easily separated allowing flexibility in the number of units needed per experiment.

Each device can also be used independently in a 2 mL microcentrifuge tube, or up to 96 samples can be dialyzed simultaneously in a standard 96-well deep-well plate using a minimal amount of buffer.

Highlights:

- **Efficient and rapid dialysis**—dialysis completed in 2–4 hours with up to 99% salt removal
- **Excellent sample recovery**—up to 90% protein recovery after dialysis
- **Ideal for small sample volume dialysis**—uses sample volumes from 10–100 µL
- **Easy to use**—complete sample loading and retrieval with a standard pipette
- **Flexible**—detachable 8-unit strips; scalable from 1 to 96 samples
- **Automation-compatible**—plate format conforms to SBS Microplate Standard

The assembled device is compatible with standard 96-well laboratory equipment and automated liquid-handling systems, making it an ideal option for high-throughput applications. When used according to the method outlined in Figure 5, the Pierce 96-well Microdialysis Plate enables the removal of low molecular weight contaminants, buffer exchange and desalting within two to four hours (Figure 4, Panel B.) with typical protein recoveries of >90%.

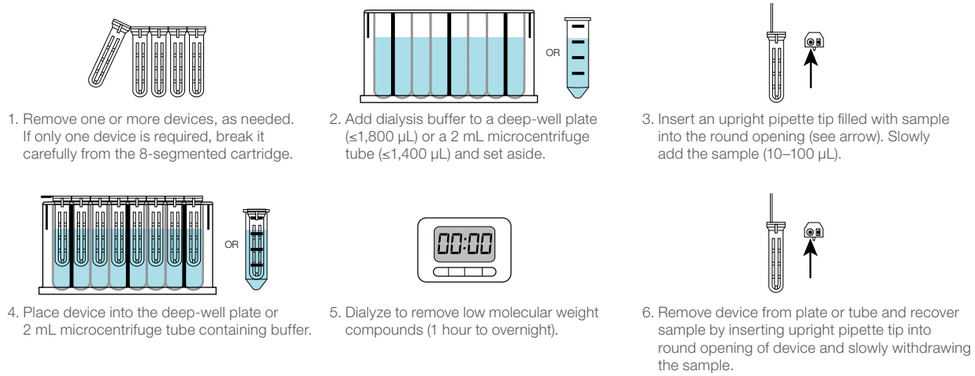
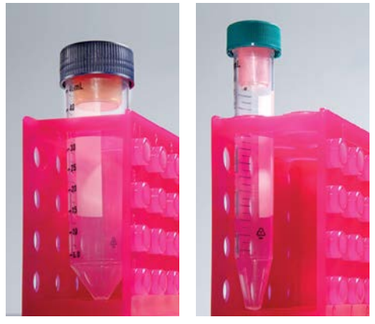


Figure 5. Protocol summary for the Thermo Scientific Pierce 96-well Microdialysis Plate.

Thermo Scientific Slide-A-Lyzer MINI Dialysis Devices

Self-contained devices for sample volumes as small as 10 µL

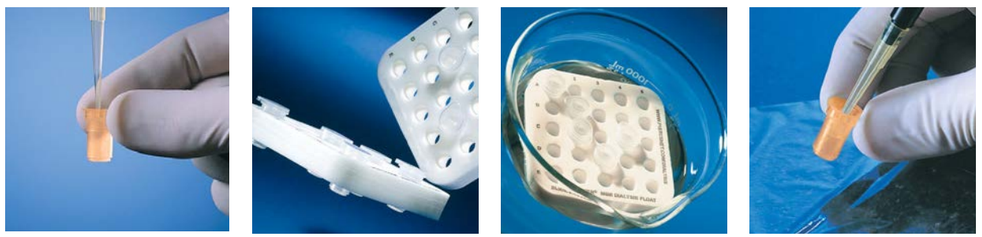


The Thermo Scientific Slide-A-Lyzer MINI Dialysis Devices have a unique cup-like design and are available in 0.1, 0.5 and 2 mL capacities. Slide-A-Lyzer MINI Dialysis Devices allow easy sample addition and removal using a standard laboratory pipette and can be used for single or arrays of samples. The self-contained, single-use devices require no syringes, centrifuge, beakers, or laborious steps. Using the Slide-A-Lyzer MINI Dialysis Devices, low molecular weight contaminant removal, buffer exchange and desalting can be accomplished within 4 to 8 hours with high protein recovery. The recommended sample volume ranges for each device are 10 µL–100 µL (0.1 mL) , 50–500 µL (0.5 mL) and 200–2,000 µL (2 mL).

Highlights:

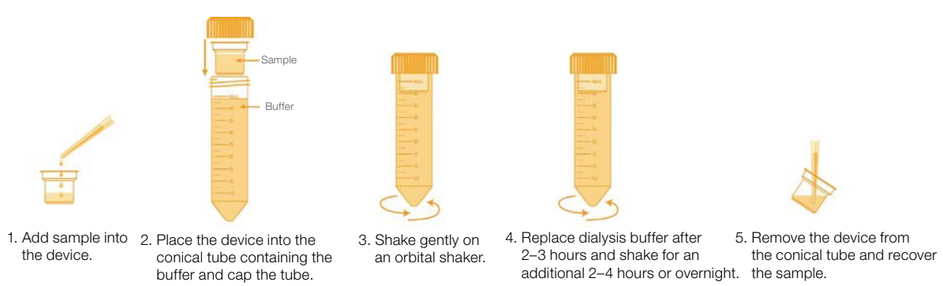
- **Excellent sample recoveries**—low-binding plastic and small membrane surface area minimize sample loss compared to filtration and resin systems
- **One-step protocol**—pipette sample into the Slide-A-Lyzer MINI Device and place in tube containing the dialysis buffer; no laborious assembly, device preparation or expensive equipment is required
- **100% leak tested**—innovative design does not permit "wicking" that can occur in homemade devices
- **Minimal dialysis buffer required**—minimizes waste

The 0.1 mL devices can be placed into a foam float during dialysis (Figure 6), and are available in MWCOs of 2K, 3.5K, 7K, 10K, or 20K. The 0.5 mL and 2 mL sizes, which are integrated into 15 mL and 50 mL capped conical tubes, respectively, are available in 3.5K, 10K and 20K MWCO. The tubes serve as dialysis reservoirs for easy and self-contained dialysis (Figure 7).



1. Apply sample with a pipette.
2. Place the Slide-A-Lyzer MINI Dialysis Device into the float.
3. Insert the float into the beaker containing the dialysate.
4. Recover sample.

Figure 6. Sample dialysis using a 0.1 mL Thermo Scientific Slide-A-Lyzer MINI Dialysis Device. The required float is sold separately.



1. Add sample into the device.
2. Place the device into the conical tube containing the buffer and cap the tube.
3. Shake gently on an orbital shaker.
4. Replace dialysis buffer after 2–3 hours and shake for an additional 2–4 hours or overnight.
5. Remove the device from the conical tube and recover the sample.

Figure 7. Sample dialysis with a 0.5 mL or 2 mL Thermo Scientific Slide-A-Lyzer MINI Dialysis Device.

Original Thermo Scientific Slide-A-Lyzer Dialysis Cassettes

Secure and convenient alternative to dialysis tubing

The original Thermo Scientific Slide-A-Lyzer Dialysis Cassettes facilitate rapid and effective dialysis for sample volumes from 100 µL to 30 mL. The cassette design maximizes surface area to sample volume ratio and provides excellent sample recovery. Unlike standard flat tubing, these innovative cassettes do not require knots or clips that can lead to leaking and sample loss, resulting in more complete sample recovery.

The Slide-A-Lyzer Dialysis Cassettes are available in five membrane MWCOs (2K, 3.5K, 7K, 10K, and 20K) and in four different sizes for dialyzing sample volumes between 0.1 and 30 mL. Slide-A-Lyzer Dialysis Cassettes can be used for a wide range of applications, including low molecular weight contaminant removal, buffer exchange, desalting, and sample concentration.

Highlights:

- **Easy to use**—no knots or clamps required; just inject sample into cassette to begin dialysis (Figure 8)
- **Fast dialysis**—flat cassette chamber with two membranes provides high surface-area-to-volume ratio that maximizes diffusion rate compared to cylindrical dialysis tubing
- **High recovery**—rectangular cassette design maximizes recovery of entire sample volume via any one of the four corner injection ports
- **Four cassette sizes**—select the cassette that best suits the sample volume
- **Sterile option**—gamma-irradiated 10K MWCO cassettes are available for applications requiring sterilized conditions

1. Insert syringe needle through the gasket via one of the corner ports. Inject the sample, withdraw the excess air and remove the syringe.
2. Attach a float buoy and dialyze. (Buoys also serve as convenient bench-top stands for the cassettes.)
3. Insert empty syringe needle at a second corner port. Inject air to expand the cassette chamber, then withdraw the dialyzed sample.

Figure 8. Thermo Scientific Slide-A-Lyzer Dialysis Cassette procedure summary.

Thermo Scientific Slide-A-Lyzer G2 Dialysis Cassettes

Maximum convenience for high-performance dialysis

Thermo Scientific™ Slide-A-Lyzer™ G2 Dialysis Cassettes provide maximum convenience, flexibility and performance for sample dialysis. These second-generation (G2) Slide-A-Lyzer Dialysis Cassettes are freestanding, self-floating and pipette-loadable. Sample loading and removal are easily accomplished by using a serological pipette or hypodermic needle and syringe (Figure 9). The built-in air chamber provides sample buoyancy and vertical orientation of the cassette during dialysis.

Highlights:

- **Easy loading**—pipette-accessible for easy sample loading and retrieval
- **Self-floating**—integrated air chambers eliminate the need for float buoys
- **Sturdy construction**—enables high sample integrity and protection
- **Superior design**—researched and tested to provide fast and consistent dialysis with maximum sample recovery
- **Multiple sizes**—five cassette capacities to optimally match 0.25 to 70 mL sample volumes
- **Versatile**—ideal for removing low molecular weight contaminants, performing buffer exchange and desalting
- **Sterile option**—gamma-irradiated 10K MWCO cassettes are available for applications requiring sterile conditions

The single-use, disposable Slide-A-Lyzer G2 Dialysis Cassettes are available in five MWCOs (2K, 3.5K, 7K, 10K, and 20K) and in five different sizes for dialyzing sample volumes between 0.25 and 70 mL. The membrane is composed of low-binding, regenerated cellulose, and the cassettes are manufactured using clean-room conditions. Select sizes of 10K MWCO Slide-A-Lyzer G2 Dialysis Cassettes are also available in packages that have been gamma-irradiated to sterilize them. Gamma-irradiated Slide-A-Lyzer G2 Dialysis Cassettes are ideal for researchers culturing cells and microorganisms; purifying viruses, DNA, and RNA; or performing sample preparation for other applications requiring sterile conditions to minimize the risk of sample contamination.

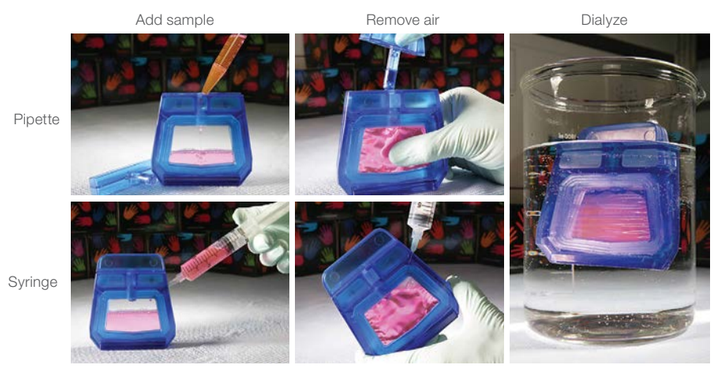


Figure 9. Thermo Scientific Slide-A-Lyzer G2 procedure summary.

Thermo Scientific Slide-A-Lyzer Dialysis Flasks

Secure and easy to handle tool for large-volume dialysis (150–250 mL)

Thermo Scientific Slide-A-Lyzer Dialysis Flasks facilitate simple and effective removal of buffer salts and small contaminants from proteins and other macromolecules in sample volumes up to 250 mL. Slide-A-Lyzer Dialysis Flasks are available in four molecular weight cutoffs (MWCOs): 2K, 3.5K, 10K, and 20K and are color-coded for easy identification. With Slide-A-Lyzer Dialysis Flasks, typical low molecular weight contaminant removal, buffer exchange and desalting can be accomplished in as few as 8 hours.

The flasks are manufactured using clean-room conditions. The flasks are constructed from two sheets of low-protein binding, regenerated cellulose membranes to help ensure maximum sample recovery and purity and contain up to 85% less plastic per volume compared to cassettes.

Highlights:

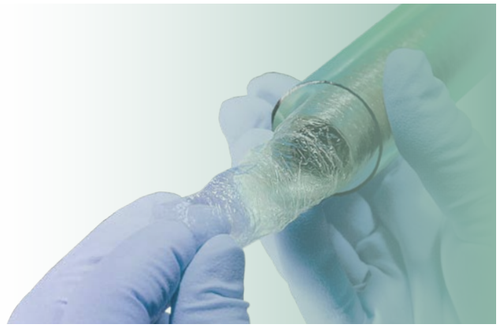
- **Easy to use**—simply pipette or pour sample into flask, replace and tighten cap, and begin dialysis
- **Fast dialysis**—flat flask chamber with two membranes provides high surface-area-to-volume ratio, enabling dialysis of a 250 mL sample in as few as 8 hours
- **High recovery**—rectangular flask design maximizes recovery of entire sample volume via opening at top of flask
- **Color-coded frames**—easily identify membrane pore size (MWCO) based on the frame color

Slide-A-Lyzer Dialysis Flasks eliminate the risk of sample loss associated with handling long lengths of slippery dialysis tubing. No knots or clips are needed to seal the units. Sample addition and removal are easily accomplished by pipetting or directly pouring the sample through the wide-mouth opening at the top of the flask (Figure 10). A simple screw cap easily and reliably seals the device.

Figure 10. Easy sample loading and recovery with Thermo Scientific Slide-A-Lyzer Dialysis Flasks. Attach supplied float-ring and hydrate membrane for 2 minutes. Pour sample into device. Remove air and cap. Dialyze for 8 hours to overnight (replace buffer after 2 and 5 hours). Pour out sample to recover.

Thermo Scientific SnakeSkin Dialysis Tubing

Easier to use than traditional flat tubing



Thermo Scientific™ SnakeSkin™ Dialysis Tubing is an easy- and ready-to-use form of traditional dialysis membrane tubing that allows desalting and buffer exchange for 10 to 100 mL samples, and it does not require presoaking or boiling prior to use. To use, simply pull out and cut off the required length of tubing, fold over one end of tubing and close with a dialysis clip, add sample at the open end, and use a second clip to close the remaining end.

Highlights:

- **Convenient**—ready-to-use, pre-wetted, pleated tube
- **High recovery**—up to 90% protein recovery
- **Speed**—dialysis is generally completed in 4 to 6 hours
- **Stability**—compatible with a variety of laboratory solutions, including acids, bases, hydrophobic solvents, and alcohols

SnakeSkin Dialysis Tubing is composed of regenerated cellulose dialysis tubing and supplied as an open, pleated (telescoped) tube. It is supplied in eight inch (20cm) sticks containing 35 feet of dialysis tubing having a 16, 22 or 35 mm circular internal diameter (I.D.). The hydrated SnakeSkin Dialysis Tubing holds ~2 to 10 mL of sample per centimeter of length. Because SnakeSkin Dialysis Tubing is made from the same type of regenerated cellulose as flat tubing, its dialysis performance matches that of conventional tubing. SnakeSkin Dialysis Tubing is available in three molecular weight cutoffs: 3.5K, 7K and 10K, with an internal diameter of 22 mm. The 3.5K and 10K MWCO membranes are also available with internal diameters of 16 and 35 mm.

Table 5. Dialysis tubing specifications and sample capacity.

Membrane MWCO* (Da)	Membrane Thickness	Tubing Diameter	Volume (mL/cm tubing)†
3.5K	1.0 mil (25 µm)	16 mm I.D.	~2.0 mL
7K	1.2 mil (30 µm)	22 mm I.D.	~3.8 mL
10K	0.9 mil (23 µm)	35 mm I.D.	~9.6 mL

*Excludes membrane length used for tube closure
†Membrane type: Regenerated cellulose

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

Protein desalting using gel filtration resins

Overview

Size exclusion chromatography (SEC) involves the chromatographic separation of molecules of different dimensions, molecular weight or size. Size exclusion chromatographic resin usually consists of small, uncharged porous particles with a range of pore sizes. Molecules are separated based on the relative abilities of molecules to penetrate into the pores. This technique is also commonly referred to as gel filtration or molecular sieve chromatography. Size exclusion chromatography is used in research and industrial applications for a wide range of applications ranging from the separation of proteins, DNA fragments and polymers.

In addition to the separation of macromolecules, SEC is also commonly used for the separation and removal of unwanted molecules from a macromolecule of interest (desalting), or exchange of the buffer for downstream applications (buffer exchange). Applications for desalting include not only the removal of salts, but also the removal of excess biotin, crosslinkers, reactive dye, radioactive labels, or other derivatization reagents from conjugation reactions. Buffer exchange is used to place a protein solution into a more appropriate buffer before subsequent applications such as electrophoresis, ion exchange and affinity chromatography, or conjugation.

How separation is achieved

Size exclusion chromatography applications for separating macromolecules based on subtle differences in size typically use resins with large and varied pore sizes in long chromatography columns. However, for buffer exchange and desalting applications, it is mainly the maximum effective pore size (exclusion limit or molecular weight cutoff (MWCO) of the resin), which determines the size of molecules that can be separated. Molecules that are significantly smaller than the MWCO penetrate into the pores of the resin, while molecules larger than the MWCO are unable to enter the pores and remain together in the void volume of the column. By passing samples through a column resin bed with sufficient length and volume, macromolecules can be fully separated from small molecules that travel a greater distance though the pores of the resin bed. No significant separation of molecules larger than the exclusion limit occurs (Figure 1).

In order for the desired macromolecules to remain in the void volume, resins with very small pore sizes must be utilized. For routine desalting and buffer exchange applications, choosing a resin with a molecular weight cutoff between 5 and 10 kDa is usually best. For other applications, such as separating peptides from full-sized proteins, resins with larger exclusion limits may be necessary. The macromolecular components are recovered in the buffer used to pre-equilibrate the gel-filtration matrix, while the small molecules can be collected in a later fraction volume or left trapped in the resin. One important feature to note when choosing a resin is that the small molecules targeted for removal must be several times smaller than the MWCO for proper separation.

Desalting vs. dialysis

Dialysis is useful for many of the same desalting and buffer exchange applications performed with gel filtration chromatography as both methods are based on similar ranges of molecular weight cutoffs, but gel filtration is faster (a few minutes vs. hours for dialysis). An additional advantage of gel filtration is the ability to remove contaminants in a relatively small volume (or left on the column), an important feature when working with toxic or radioactive substances. Dialysis, on the other hand, is much less dependent on sample size as related to device format.

For dialysis applications, achieving a high-percentage sample recovery and molecule removal is generally straightforward with little optimization needed. For gel filtration applications it is important to select a column size and format that is suitable for your sample.

Gravity-flow, or drip, columns use head-pressure from a buffer chase to push the sample through the gel filtration matrix. The sample is loaded into the top of an upright column and allowed to flow into the resin bed. The sample is then chased through the column by adding additional buffer or water to the top of the column. During this process, small fractions are typically collected and tested for the macromolecules of interest. As an alternative to fraction collection, a single fraction equal to the full exclusion volume of the column is collected regardless of the sample volume. This eliminates the time and monitoring associated with fraction collecting; however, this can result in significant dilution of the sample depending on the sample volume. Gel filtration formats for smaller volumes include gravity-flow columns, chromatography cartridges, centrifuge columns and centrifuge plates.

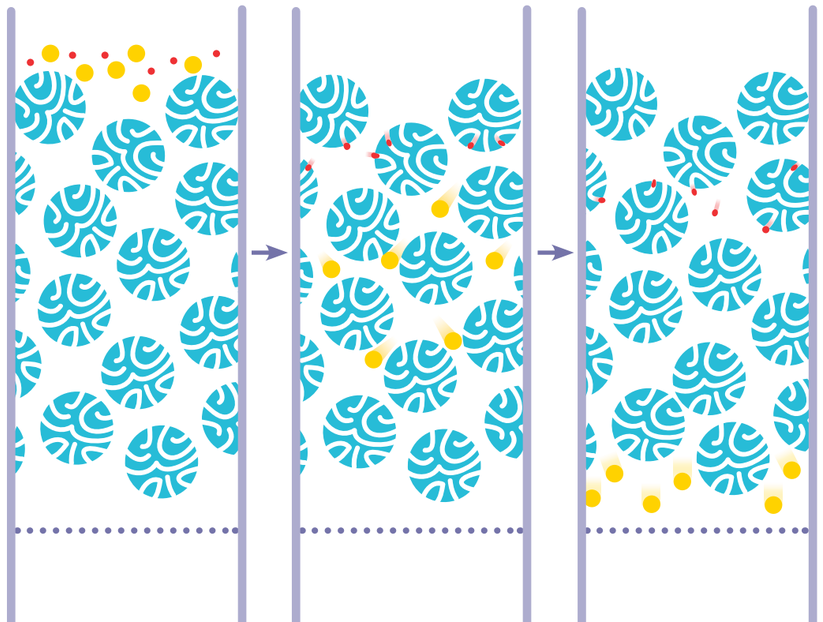


Figure 1. Passage of a protein sample through a column of porous resin facilitates buffer exchange and desalting. Small molecules in the original sample (red) enter the bead pores, thereby taking a longer and slower path through the column than the protein (yellow). As a result the protein separates from the original buffer salts and exchanges into the column buffer.

Resin performance data

Optimized products for improved protein recovery and faster desalting

To eliminate sample dilution and the collecting and monitoring of fractions, centrifuge-column or plate-based gel filtration, also referred to as spin desalting methods, are commonly used. Spin desalting is unique in that a centrifuge is used to first remove the resin's void volume of liquid, followed by sample addition and centrifugation. After centrifugation the macromolecules in the sample have moved through the column in approximately the same initial volume, but the small molecules have been forced into the pores of the resin and replaced by the buffer that was used to pre-equilibrate the gel-filtration matrix (Figure 2). Spin formats eliminate the need to wait for samples to emerge by gravity flow and require no chromatography system, allowing for multiple sample processing simultaneously. However, due to the lack of a chase buffer, spin column methods have historically suffered from sample loss, particularly at low protein concentrations, and the sample volume they could be used with were limited.

Thermo Scientific Zeba desalting products contain a unique resin 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. Two MWCOs (7K and 40K MWCO) are available to optimize for larger or smaller proteins and/or contaminants. Multiple formats can accommodate the different needs for sample volumes, automation and throughput.

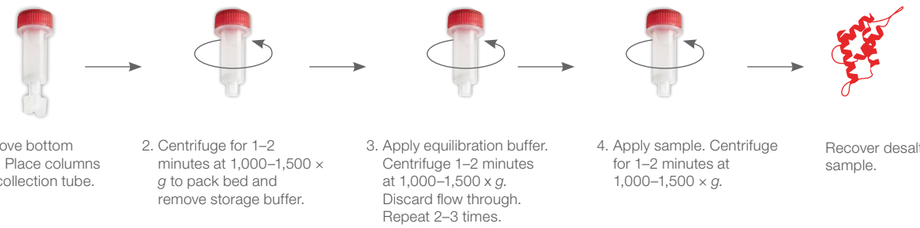


Figure 2. Protocol for desalting with Thermo Scientific™ Zeba™ Spin Desalting Columns.

Table 1. Thermo Scientific™ Zeba™ resin selection guide by protein recovery and small molecule removal.

	7K		40K	
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 Recommended + = Good ++ = Better +++ = Recommended

Table 2. Thermo Scientific Zeba Spin Desalting Columns provide exceptional protein recovery over a wider range of sample concentrations and volumes compared to alternative products. Desalting columns were equilibrated with a final buffer containing 25 mM Tris, 25 mM NaCl at pH 7.5. Samples were diluted in 25 mM Tris, 500 mM NaCl at pH 7.5 and then desalted into the final buffer. All samples were processed according to the manufacturers' recommended instructions, but load volume may exceed recommended ranges (see below). There were no significant differences in salt concentration between any vendor when used within the recommended ranges. Protein concentration was determined using the Thermo Scientific™ Pierce™ BCA Protein Assay (Cat. No. PI23227). Recoveries with lysate are typically slightly lower than seen with individual proteins due to the loss of small molecular weight compounds and lysis contaminants that can influence protein assay results.

	Sample volume	7K Zeba Spin Columns, 0.5 mL	GE SpinTrap G-25	Bio-Rad Micro Bio-Spin 6 Columns	Sample volume	7K Zeba Spin Columns, 10 mL	GE PD-10 Columns	
Resin bed volume		550 µL	500 µL	700 µL		10 mL	8.3 mL	8.3 mL
Method		Spin	Spin	Spin		Spin	Spin	Drip
		% Protein recovery				% Protein recovery		
BSA 0.04 mg/mL	40 µL	70–80%	<10%	<10%	1.5 mL	75–90%	20–30%	40–50%***
	80 µL	80–95%	<10%	<10%	2.5 mL	75–90%	30–40%	45–55%***
	120 µL	>90%	<10%	<20%	3.5 mL	75–90%	60–70%*	55–65%*
BSA 0.2 mg/mL	40 µL	70–80%	<15%	25–35%	1.5 mL	>90%	60–70%	>90%***
	80 µL	80–95%	30–45%	45–60%	2.5 mL	>90%	70–80%	85–95%***
	120 µL	>90%	55–70%	65–80%	3.5 mL	>90%	75–85%**	75–85%**
BSA 1 mg/mL	40 µL	80–95%	45–60%	70–80	1.5 mL	>90%	80–90%	>90%***
	80 µL	>90%	60–75%	80–90	2.5 mL	>90%	80–90%*	>90%***
	120 µL	>90%	75–90%	>90%	3.5 mL	>90%	80–90%*	85–95%*
HeLa lysate 0.2 mg/mL**	40 µL	45–55%	<10%	<15%	1.5 mL	70–85%	55–65%	>90%***
	80 µL	60–70%	15–25%	30–40%	2.5 mL	70–85%	65–75%	80–95%***
	120 µL	70–80%	45–55%	50–60%	3.5 mL	80–95%	70–80%*	75–85%*
HeLa lysate 1 mg/mL**	40 µL	65–75%	45–55%	45–55%	1.5 mL	80–95%	80–90%	>90%***
	80 µL	75–85%	60–70%	70–80%	2.5 mL	80–95%	80–95%	>90%***
	120 µL	80–90%	70–80%	80–90%	3.5 mL	85–95%	80–95%*	85–95%*

*Note: The recommended sample volumes for the columns tested above would result in insufficient salt removal. They are included only to help demonstrate the effect of a small volume on protein recovery.

**Note: Recoveries with lysate are typically slightly lower than those seen with individual proteins due to the loss of small molecular weight compounds and lysis contaminants that can influence protein assay results.

***Note: Sample diluted during processing and recovered in 3.5 mL final volume. Protein recovery determined on total final volume.

Protein recovery compared to other vendors

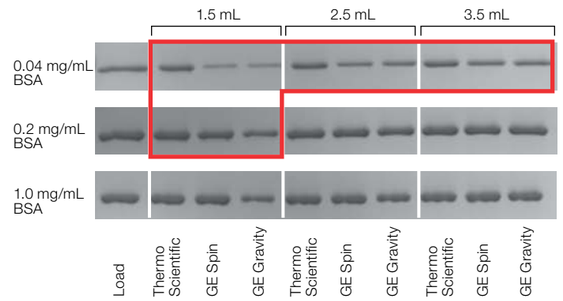


Figure 3. Thermo Scientific Zeba Spin Desalting Columns provide a high protein recovery while providing minimal sample dilution over a wider range of sample concentrations and volumes compared to alternative products. Zeba Spin Desalting Columns, 10 mL (7K MWCO) (Cat. No. PI89893) and GE PD-10 Columns 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 Load Control; all other desalted samples were loaded in the gel at the same volume as the Load 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.

Table 3. Comparison of protein recovery, from the 7K MWCO and 40K MWCO, 0.5 mL Thermo Scientific Zeba Spin Desalting Columns.

			Typical % recovered from 100 µL sample	
Recovery of	Concentration loaded	Size of molecule	7K Zeba Column	40K Zeba Column
Ubiquitin	0.5 mg/mL	8.7 kDa	75%	60%
α-Lactalbumin	1.0 mg/mL	14.1 kDa	85%	75%
Soybean Trypsin Inhibitor	0.5 mg/mL	20.1 kDa	85%	65%
Carbonic Anhydrase	0.5 mg/mL	29 kDa	90%	75%
Ovalbumin	0.5 mg/mL	44 kDa	90%	85%
Bovine Serum Albumin	0.5 mg/mL	66 kDa	>90%	>90%
Human IgG	0.5 mg/mL	150 kDa	>90%	>90%

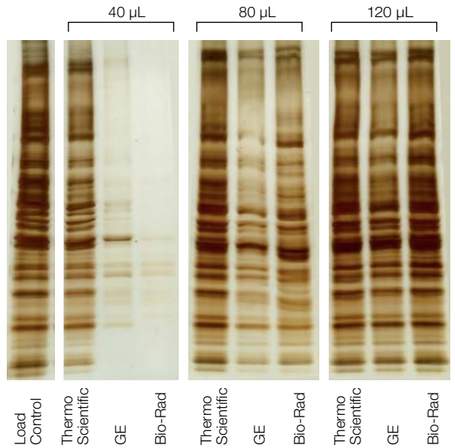
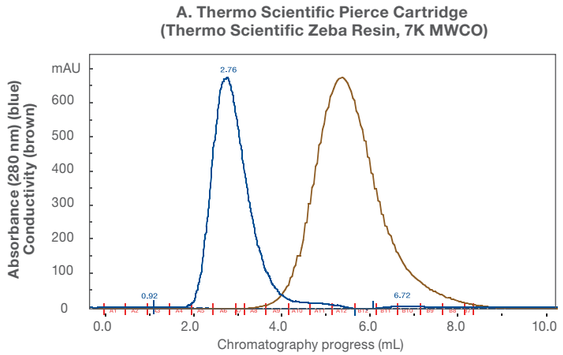


Figure 4. Better performance with Thermo Scientific Zeba Spin Desalting Columns. Zeba Spin Desalting Columns provide a high protein recovery while providing minimal sample dilution over a wider range of sample concentrations and volumes compared to alternative products. Zeba Spin Desalting Columns, 0.5 mL (7K MWCO) (Cat. No. PI89882), GE SpinTrap G-25 and Bio-Rad Micro Bio-Spin 6 spin columns were used to desalt 40, 80 and 120 µL samples of HeLa lysate at a concentration of 0.2 and 1 mg/mL. Desalting was performed according to the manufacturers' recommended protocols. Protein recovery was analyzed by SDS-PAGE. For each electrophoresis gel, an aliquot of starting sample equal to 1 µg of HeLa lysate was loaded in Lane 1 as the Load Control; all other desalted samples were loaded in the gel at the same volume as the Load Control. Differences in intensity between lanes are a combination of protein recovery and sample dilution caused by desalting.

Table 4. Comparison of small molecule removal, for the 7K MWCO and 40K MWCO, 0.5 mL Thermo Scientific Zeba Spin Desalting Columns.

			Typical % removed from 100 µL sample	
Removal of	Concentration loaded	Size of molecule	7K Zeba Column	40K Zeba Column
NaCl	1 M	58.44 Da	>99%	>99%
Dithiothreitol	0.5 M	154 Da	99%	99%
Sulfo-NHS-LC-Biotin	0.27 mM	557 Da	85%	85%
Dy549 Dye	0.2 mM	1,026 Da	75%	90%
Bacitracin	0.5 mg/mL	1,200 Da	75%	95%
Vitamin B12	0.5 mg/mL	1,386 Da	85%	95%



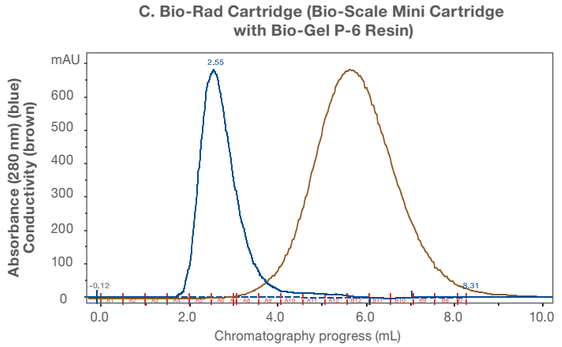


Figure 5. Efficient salt removal and protein recovery with Thermo Scientific Zeba Desalting Chromatography Cartridge. Bovine serum albumin (1 mg) in 1 M NaCl was applied to 5 mL Zeba Desalting Cartridge (Cat. No. 89935) (A) at a flow rate of 5 mL/minute. Cartridge profile shows isocratic elution of BSA (blue) and NaCl detected by conductivity (brown). Greater than 95% of the BSA was recovered and more than 95% of the salt was removed. Results for the Pierce Cartridge **(A)** were essentially identical to those obtained with the more expensive GE Healthcare **(B)** and Bio-Rad **(C)** products.

Selecting the right format for sample processing

Thermo Scientific Zeba desalting products contain proprietary high-performance resins with exceptional desalting and protein-recovery characteristics compared to other commercially available spin desalting media. Even very dilute protein samples can be successfully processed with high levels of protein recovery and greater than 95% retention (removal) of salts and other small molecules. Zeba desalting products rapidly process sample volumes ranging from 10 µL to 4 mL. The combination of the unique resin and easy-to-use column, plate and cartridge formats help ensure maximum protein recovery in minimum time.

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

Unlike the limited offerings of other suppliers, Zeba Spin Desalting Columns are available in a wide range of formats to match a variety of applications (Table 5). Our broad portfolio of high-performance devices for desalting and buffer exchange provide easy handling, rapid processing and exceptional recovery for sample volumes between 2- and 4,000 µL. In addition, two size-exclusion resin options (7K and 40K MWCO) are available. The 7K Zeba Desalting Resin is recommended for removing molecules <800 Da from macro molecules greater than 7 kDa. The 40K Zeba Desalting Resin is recommended for removing molecules <1,500 Da from macro molecule greater than 30–40 kDa. Salt removal is typically 95–100%. Examples of protein-specific recovery, contaminant removal data, and benchmarks versus similar products from other suppliers are shown on pages 17–20.

Table 5. Thermo Scientific Zeba desalting products selection guide by format

	Zeba Spin Desalting Columns	Zeba 96-well Spin Plates	Zeba Chromatography Columns
			
Sample volume range	2 µL–4 mL	20–100 µL	15–250 µL or 100 –1,500 µL
Bed volume range	75 µL–10 mL	550 µL	1 mL or 5 mL
Number of devices with different volume capacities	5	1	2
Molecular weight cutoffs (MWCOs)	7K, 40K	7K, 40K	7K
Automation compatible?	No	Yes	No
FPLC compatible?	No	No	Yes

Zeba Spin Desalting Columns

Zeba Spin Desalting Columns are made of low protein binding polypropylene and are compatible with a wide range of standard laboratory instruments and consumables. The Zeba Spin Desalting Columns and Plates are designed to be compatible with most swinging-bucket or fixed-angle bench or floor model centrifuges; however, proper head clearance should be verified before use.

Table 6. Thermo Scientific Zeba desalting products selection guide by format, recommended sample volume, and column schematics.

Format	Micro spin column	0.5 mL spin column	2 mL spin column	5 mL spin column	10 mL spin column
Resin bed	75 µL	0.5 mL	2 mL	5 mL	10 mL
Sample volume (7K MWCO)	2–12 µL	30–130 µL	200–700 µL	500–2,000 µL	700–4,000 µL
Sample volume (40K MWCO)	5–14 µL	70–200 µL	200–900 µL	300–2,000 µL	1,000–4,000 µL

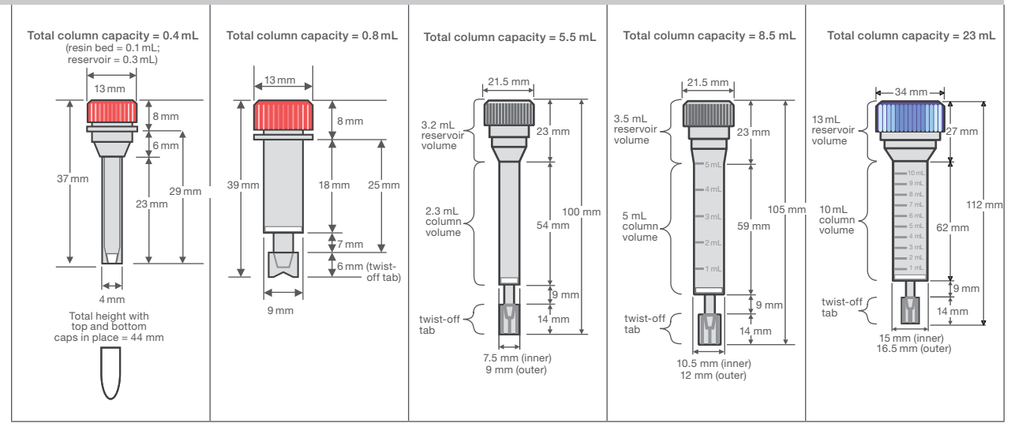
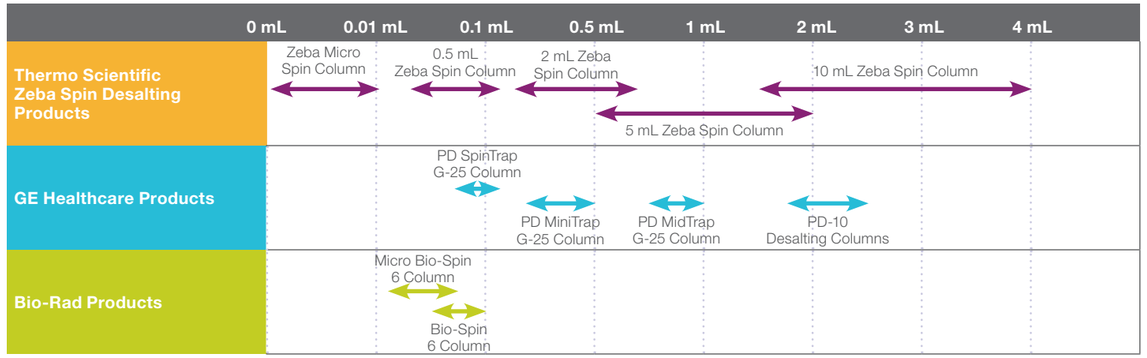


Table 7. Comparison of recommended sample volume capacities of common spin desalting products.



Zeba Spin Desalting Plates

The pre-packed Thermo Scientific™ Zeba™ 96-well Spin Desalting Plates do not require resin hydration or dispensing and provide the same high protein recovery as Zeba Spin Desalting Columns. Each Zeba Spin Desalting Plate can process up to 96 samples (20 to 100 µL) in as few as 10 to 20 minutes. A collection plate is provided with each filter plate.

Table 8. Common specifications of Thermo Scientific Zeba 96-well filter plates.

Plate dimensions (l x w x h)†	127.76 x 85.5 x 45 mm ± 0.25 mm
Well depth	27.11 ± 0.10 mm
Well diameter	7 ± 0.10 mm
Well off-set	9 mm
Well volume	800 µL
Plate material	polypropylene
Filter material	polyethylene
Filter pore size	20 µm
Collection plate maximum volume	150 µL
Max centrifuge speed	up to 1,000 x *g*
Suggested balance plate	use Cat. No. PI45205

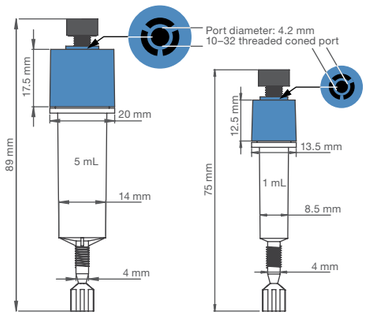
† Plate height includes collection plate for total stack height.

Zeba Desalting Chromatography Cartridges

Thermo Scientific™ Zeba™ Desalting Chromatography Cartridges are available pre-packed in 1 mL and 5 mL sizes. They can be regenerated for multiple uses and efficiently process samples from 50 to 1,500 µL. Zeba Desalting Chromatography Cartridges can be processed manually or by automated liquid chromatography (LC) systems. The cartridges attach directly to the ÄKTA™ FPLC™ system from GE Healthcare or other FPLC systems without additional connectors. Cartridge products include an accessory pack of tubing fittings and Luer-Lok™ fittings that provide compatibility with the other popular LC systems and manual syringe processing. They are available only in the 7K MWCO resin.

Table 9. Thermo Scientific Zeba Desalting Chromatography Cartridge properties. Recommended and maximum flow rates are general; values differ slightly for individual products.

Feature	1 mL Cartridge	5 mL Cartridge
Dimensions	0.7 x 2.7 cm	1.3 x 3.8 cm
Desalting flow rate (Maximum)	0.2 to 1 mL/minute (3 mL/minute)	1 to 5 mL/minute (8 mL/minute)
Affinity flow rate (Maximum)	0.1 to 1 mL/minute (4 mL/minute)	0.5 to 2 mL/minute (5 mL/minute)
Maximum pressure	0.3 MPa (43 psi or 3 bar)	0.3 MPa (43 psi or 3 bar)
Cartridge material	polypropylene	polypropylene
Frit material	polyethylene	polyethylene



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

Detergent removal using chromatography resins

Overview

Detergents are a class of molecules whose unique properties enable manipulation (disruption or formation) of hydrophobic and hydrophilic interactions among molecules in biological samples. In life science applications, detergents are used for cell lysis, protein solubilization and denaturation, or to reduce background in certain applications.

The detergents and surfactants used to prepare protein and peptide samples can interfere with analysis by ELISA, isoelectric focusing and mass spectrometry (MS). Removing detergents from peptide samples is especially challenging and critical for MS analysis because even low concentrations of detergents will contaminate instruments and interfere with column binding, elution and peptide ionization.

Detergent removal can be attempted in a number of ways. Acetone or acid precipitation can be used for proteins (not peptides) and generally has poor recovery. Dialysis is effective for removal of detergents that have very high Critical Micelle Concentration (CMC) and/or small aggregation numbers, such the n-octyl glucoside formulations. Detergents with low CMCs and large aggregation numbers cannot be dialyzed because most of the detergent molecules will be in micelles that are too large to diffuse through the pores of the dialysis membrane; only excess monomer can be dialyzed. Ion exchange chromatography using appropriate conditions to selectively bind and elute the proteins of interest is another effective way to remove detergents. For peptides, ion exchange can be used to bind and remove selective detergents, but only if they are anionic or cationic detergents, such as SDS. Sucrose density gradient separation also can be used. However, all these methods can be somewhat labor- and/or time-intensive or detergent-specific.

Improved tools to quickly and efficiently remove detergents

The Thermo Scientific™ Pierce™ detergent removal products contain a proprietary resin that specifically bind a wide variety of detergents and surfactants that are commonly used in protein extraction and biological sample preparation. The spin column format provides a convenient and rapid method for removing interfering detergents from protein and peptide solutions before downstream analysis by MS and other techniques. Samples can be processed in as little as 15 minutes.

Resin performance data

Detergent removal efficiency and protein recovery

Table 1. Results using Thermo Scientific™ HiPPR™ Detergent Removal Resin. Each column and plate well contained ~550 µL of detergent-removal resin slurry and 0.1 mL of sample. Similar results were obtained with both process formats.

Process format†	Detergent	Detergent concentration (%)	Detergent removal (%)	BSA recovery (%)
0.5 mL Spin Column	Sodium deoxycholate	5	99	100
	Octyl glucoside	5	99	90
	Octyl thioglucoside	5	99	95
	Lauryl maltoside	1	98	99
	Triton X-114	2	95	100
	Brij-35	1	99	97
	Tween 20	0.25	99	87
96-well Spin Plate	SDS	5	99	89
	Triton X-100	4	99	100
	NP-40	1	95	100
	CHAPS	5	99	100

Table 2. Results using standard Thermo Scientific™ Pierce™ Detergent Removal Spin Column, 0.5 mL. Detergent removal efficiency and protein recovery. BSA sample (25–200 µL) + detergent in 0.15 M NaCl, 0.05% sodium azide was mixed with equal volume of detergent removal resin (2x volume for CHAPS removal) and processed as shown in the protocol (page 29).

Detergent	Sample volume (µL)	Protein quantity (µg)	Detergent removal (%)	Protein recovery (%)
SDS (1%)	25	0.375	>99	98
	50	0.75	>99	97
	100	1.5	>99	100
	200	3.0	>99	100
Triton X-100 (1%)	25	0.375	>95	82
	50	0.75	>95	86
	100	1.5	>95	86
	200	3.0	>95	93
NP-40 (0.75%)	25	0.375	95	90
	50	0.75	96	94
	100	1.5	97	91
	200	3.0	97	97
CHAPS (1%)	25	0.375	95	64
	50	0.75	97	70
	100	1.5	98	78
	200	3.0	98	75

LC/MS analysis of digested BSA sample

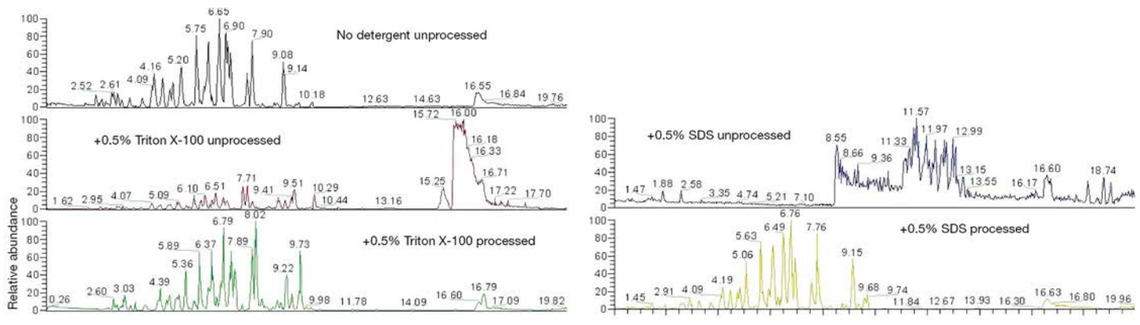


Figure 1. Results using HiPPR Detergent Removal Resin. BSA (100 µg/mL) tryptic digests were prepared without detergent, in the presence of 0.5% Triton X-100 or spiked with 0.5% SDS following enzymatic digestion. Samples (0.1 mL) containing detergent were processed with the HiPPR Detergent Removal Resin and compared to unprocessed or detergent-free samples by LC-MS/MS. Results demonstrate that detergent removal is effective and produces results similar to those observed for samples containing no detergent.

LC/MS analysis of digested peptide

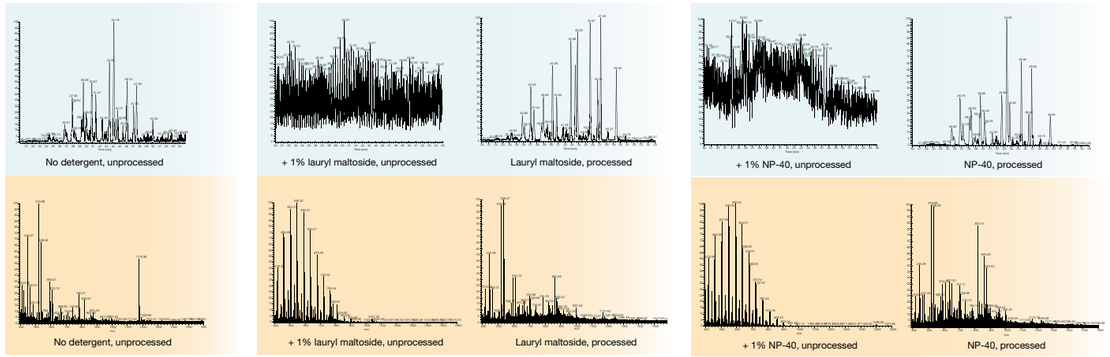


Figure 2. Results using standard Pierce Detergent Removal Spin Column, 0.5 mL. Tryptic digests (0.1 mL, 100 µg) containing detergent were each processed through 0.5 mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. Top row: Base peak LC-MS chromatograms. Bottom row: Integrated mass spectra. Similar results were produced for Brij™-35 detergent, octyl glucoside, octyl thioglucoside, and SDS (data not shown).

Peptide identification results

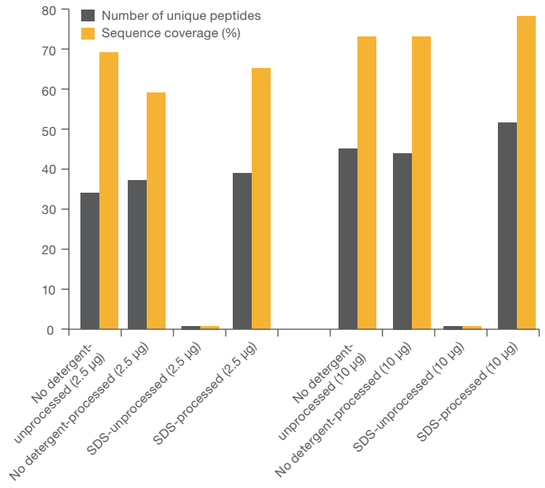


Figure 3. Results using HiPPR Detergent Removal Resin. BSA (25 and 100 µg/mL) was digested in the presence and absence of detergents and the samples were processed for LC-MS/MS analysis. Effective detergent removal resulted in greater peptide identification and high MASCOT™ scores.

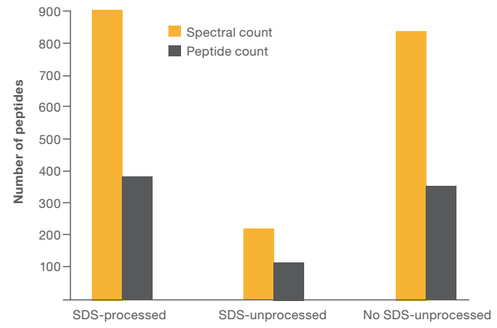


Figure 4. Results using Pierce Detergent Removal Spin Columns, 0.5 mL. A tryptic digest of HeLa cell lysate (0.1 mL, 100 µg) containing 1% SDS was processed through 0.5 mL of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. The processed sample allowed similar numbers of identified peptides as digests containing no SDS. Peptide identification is greatly reduced in sample containing SDS. Effective detergent removal enables greater peptide identification.

Selecting the right format for sample processing

The Thermo Scientific Pierce Detergent Removal Resins are provided in convenient spin column or plate formats that quickly and efficiently remove ionic, nonionic and/or zwitterionic detergents from protein or peptide samples to improve compatibility with downstream applications. Two formulations are available that are optimized to remove detergents from peptide samples with different concentration ranges. The HiPPR (High Protein and Peptide Recovery) products are recommended for peptide samples </= 100 µg/mL. The standard Pierce Detergent Removal Resin products are ideal for peptide samples >100 µg/mL.

Highlights:

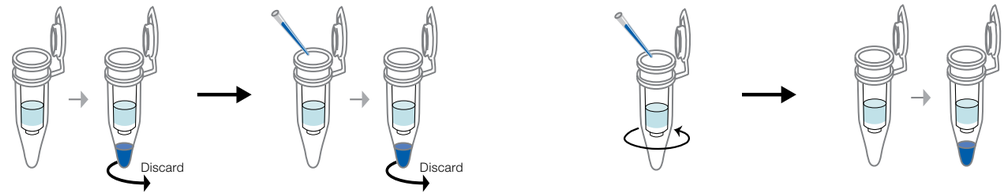
- **High performance**—removes detergent with >90% recovery and no sample dilution
- **Versatile**—effectively removes a wide variety of detergents from peptide or protein samples
- **Optimized**—separate formulations for samples with peptide concentrations </= or >100 µg/mL
- **Flexible**—available in various formats, including spin columns, 96-well spin plates, and loose resin
- **Convenient**—simple method that improves MS peptide coverage

Table 3. Detergent removal product selection guide

	HiPPR Detergent Removal Spin Columns	HiPPR Detergent Removal Spin Kit	HiPPR Detergent Removal Spin Plates	Detergent Removal Spin Columns (4 sizes)	Detergent Removal Resin	Detergent Removal Spin Plates
						
Sample volume range(s)	100 µL	25–200 µL	100 µL	10–25 µL 25–100 µL 150–500 µL 500–1,000 µL	10–2,500 µL	20–100 µL
Recommended peptide sample concentration range	1–100 µg/mL	1–100 µg/mL	1–100 µg/mL	>100 µg	>100 µg	>100 µg
Format	Pre-filled spin columns	5 mL resin + 0.8 mL empty spin columns	96-well pre-filled spin plate	Pre-filled spin columns	10 mL resin	96-well pre-filled spin plate
Resin bed volume(s)	0.1 mL	0.025–0.2 mL	0.1 mL	0.125 mL 0.5 mL 2 mL 4 mL	0.125 mL–10 mL	0.55 mL

Spin columns, loose resin and kits

The HiPPR Detergent Removal Resin is available in a pre-dispensed 0.1 mL format or as a kit with bulk resin and empty spin columns for customizing filling and processing. The Pierce Detergent Removal Resin is available in four convenient pre-packed column sizes for quick and easy sample processing; simply remove storage buffer, wash resin with equilibration buffer, add sample, incubate, and obtain detergent free sample upon final centrifugation. The resin is also available in a loose resin (10 mL pack size) for customized applications or columns.

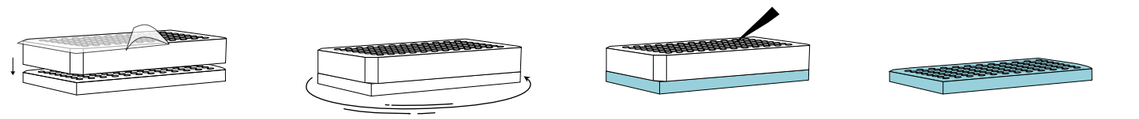


1. Centrifuge for 1 minute at 1,500 x *g* to remove the storage buffer.
2. Add 0.4 mL equilibration buffer, centrifuge at 1,500 x *g* for 1 minute and discard the flow-through. Repeat two additional times.
3. Add detergent-containing sample (25–100 µL) and incubate for 2 minutes at RT.
4. Centrifuge at 1,500 x *g* for 2 minutes to collect the detergent-free sample for downstream applications.

Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Columns (0.5 mL).

96-well spin plates

The pre-packed Thermo Scientific™ HiPPR™ and Pierce™ 96-well Detergent Removal Spin Plates do not require resin hydration or dispensing, and offer the same high protein and peptide recovery as the spin column format. Each plate can process up to 96 samples simultaneously, using 25 to 100 µL of sample per well.



1. Remove the bottom seal and stack the detergent-removal plate on top of a wash plate. Remove the top seal and centrifuge.*
2. Add 300 µL of buffer to each well and centrifuge. Discard the flow-through. Repeat this step two times.
3. Stack the detergent-removal plate on top of a sample-collection plate. Apply sample and incubate at room temperature for 2 minutes. Centrifuge to remove detergent.
4. Recover the detergent-free sample for downstream analysis.

*Centrifugations are performed for 2 minutes at 1,000 x *g*.

Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Plates.

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

Protein concentration using ultrafiltration membranes

Overview

Traditional dialysis or gel filtration is effective for contaminant removal and buffer exchange, but additional sample processing is often required to concentrate dilute protein samples. Following dialysis, the regenerated cellulose tubing or device can be submerged into a hygroscopic reagent (such as polyethylene glycol) to draw the solution across the membrane and concentrate the sample. This combined dialysis and dehydration method is gentle but time intensive. The preferred method for the rapid concentration and buffer exchange (diafiltration) of small to mid-volume protein samples is using centrifugal concentrators containing ultrafiltration membranes.

Dilute protein solutions can be concentrated using chemicals such as ammonium sulfate, trichloroacetic acid (TCA) or potassium chloride/sodium dodecyl sulfate, or through freeze-drying (lyophilization). However, chemical protein precipitation may reduce protein activity and yield. In addition, these agents are contaminants, often requiring removal for downstream applications. Although lyophilization does not create these issues, it is more time consuming than other methods.

The preferred method for the rapid concentration and buffer exchange (diafiltration) of small to mid-volume protein samples is using centrifugal concentrators containing ultrafiltration membranes. During concentration, both liquid (buffers) and low molecular weight solutes are forced through the membrane 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) as the reagent is forced across the membrane to the opposite side. For buffer exchange, the retentate is diluted to the original volume with exchange buffer and centrifuged. This can be repeated until the desired level of exchange or desalting has been achieved.

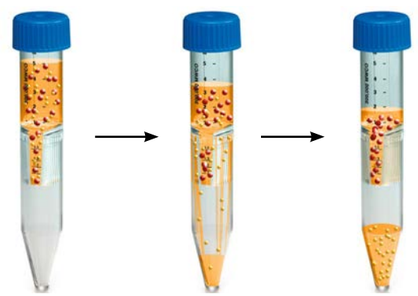


Figure 1. Overview of sample processing steps using Thermo Scientific™ Pierce™ Protein Concentrators.

Selecting the right membrane

The most commonly used membranes for protein concentration devices are polyethersulfone (PES), regenerated cellulose, and cellulose triacetate (CTA). PES has a uniform surface devoid of hydrophobic or hydrophillic interactions and offers excellent protein recovery for most solutions. PES membranes exhibit low fouling characteristics, exceptional flux and broad pH range. Regenerated cellulose membranes are highly hydrophillic and may show higher protein recovery than other membranes when processing very dilute solutions. Regenerated cellulose is resistant to autoclaving, can be cleaned and re-used, and has extended chemical resistance. Cellulose triacetate (CTA) membranes have high hydrophilicity and very low nonspecific binding that characterizes this membrane. Cast without any membrane support that could trap or bind passing micro solutes, these membranes are preferred for sample cleaning and protein removal and when high recovery of the filtrate solution is of primary importance.

Devices for fast concentration and high protein recovery

Thermo Scientific Pierce Protein Concentrators were designed to provide rapid sample processing and high recovery over a wide range of sample volumes (100 µL–100 mL). These easy-to-use devices contain a high performance PES membrane and are available in multiple sizes to process different sample volumes efficiently and effectively. A range of molecular weight cutoffs–3K, 5K, 10K, 30K, and 100K MWCOs are available to accommodate proteins as small as 6K or greater than 600K MWCO.

Table 1. Comparison of protein recovery for the Thermo Scientific Pierce Protein Concentrators based on molecular weight cutoff (3K, 10K, 30K, and 100K MWCO)

Protein	3K MWCO	10K MWCO	30K MWCO	100K MWCO
	Percent Recovery (%)			
Aprotinin, 6.5 kDa (0.25 mg/mL)	97		5	
Ubiquitin, 8.5 kDa (0.1 mg/mL)	93			
Cytochrome C, 12 kDa, (0.25 mg/mL)	95	92	6.5	
Carbonic anhydrase, 30 kDa (0.25 mg/mL)		95	95	
Ovalbumin, 45 kDa, (0.25 mg/mL)		94	96	
BSA, 66 kDa, (0.25 mg/mL)	98	98	(0.1 mg/mL) = 96 (0.25 mg/mL) = 98	23
IgG, 150 kDa, (0.25 mg/mL)			94	98
Fibrinogen, 340 kDa, (0.25 mg/mL)				92
Ferritin, 440 kDa, (0.1 mg/mL)				97
Apoferritin, 480 kDa, (0.4 mg/mL)				85
Thyroglobulin, 660 kDa (0.05 mg/mL)				(0.05 mg/mL) = 98 (0.25 mg/mL) = 98

Membrane performance data

Protein recovery compared to other vendors

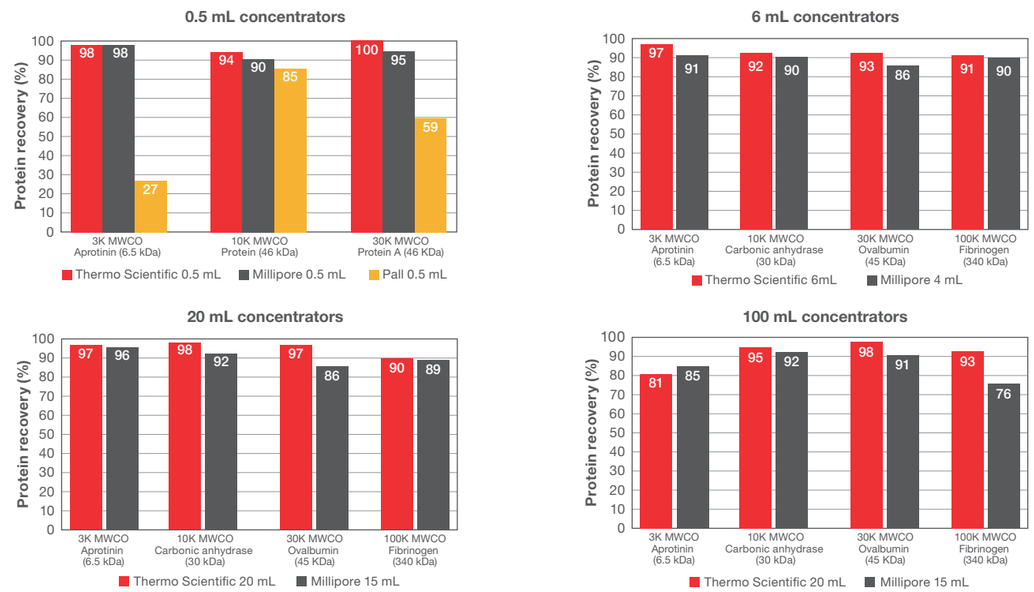


Figure 2. Comparison of protein recovery between Pierce Concentrators (using 3K, 5K, 10K, 30K, or 100K MWCO) and other vendors for 0.5 mL, 6 mL, 20 mL, and 100 mL concentrators. Samples of different protein solutions were centrifuged in Pierce Protein Concentrators and other suppliers' concentrators according to 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*) until a greater than 15- to 30-fold decrease in sample volume was achieved. Protein concentration was measured by either Pierce BCA Protein Assay (0.5 mL concentrators only) or absorbance at A_{280}.

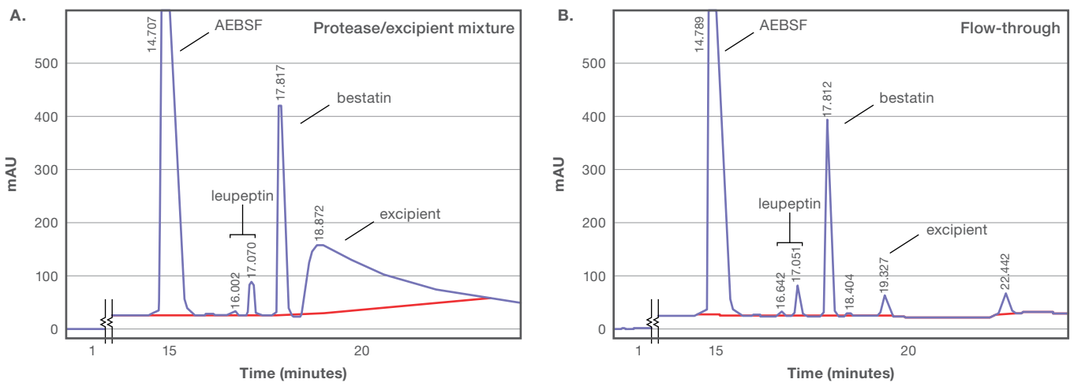


Figure 3. Separation of polymers from small molecules using Pierce Protein Concentrators PES, 100K MWCO, 0.5 mL. The excipient, sodium starch glycolate (MW >500,000 to 11,000,000), was separated from a mixture of protease inhibitors leupeptin (MW 475.6), AEBSF (MW 239.69), and bestatin (MW 345) using the Pierce Protein Concentrator PES, 100K MWCO, 0.5 mL. The excipient and the inhibitors with excipient were dissolved in 0.15 M NaCl. Half of the volume was loaded into the concentrator after pre-wetting the membrane with 0.15 M NaCl. Samples were centrifuged for 10 minutes at 13,000 x *g*. The flow-through was collected and compared to the non-concentrated controls using reverse-phase HPLC. HPLC conditions: column = Synergi 4u Hydro-RP 80A, Gradient Buffer A = 0.1% TFA in water; Gradient Buffer B = 0.1% TFA in acetonitrile. **Panel A.** HPLC chromatogram of the excipient and protease inhibitor mixture. **Panel B.** HPLC chromatogram of the mixture after removal of the excipient using the concentrator. Peaks are identified and labeled.

Flux rates for different MWCOs and sizes

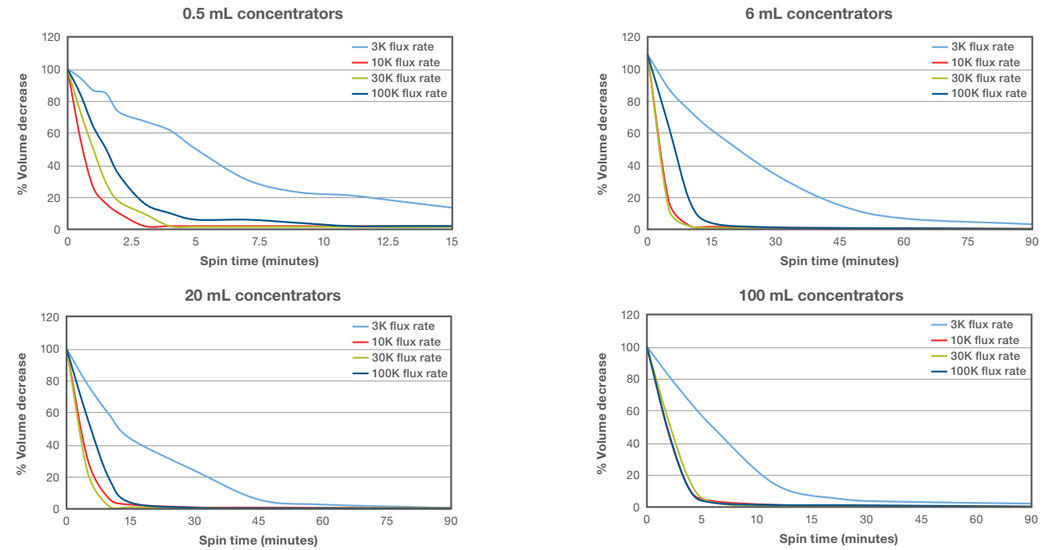


Figure 4. Comparison of flux rates for different MWCOs using 0.5 mL, 6 mL, 20 mL, and 100 mL concentrators. Samples of protein for each MWCO (Cytochrome C, 12 kDa (3K); Ovalbumin, 45 kDa (10K); BSA, 66 kDa (30K); and Thyroglobulin, 660 kDa (100K), at approximately 0.25 mg/mL starting concentrations) were centrifuged in Pierce Protein Concentrators to determine the rate at which >90% protein is concentrated. **0.5 mL concentrators:** 3 minutes for 10K, 30K, and 100K and 15 minutes for 3K. **6 mL concentrators:** 15 minutes for 10K, 30K, and 100K, and 90 minutes for 3K. **20 mL concentrators:** 15 minutes for 10K, 30K, and 100K, and 60 minutes for 3K. **100 mL concentrators:** 15 minutes for 10K, 30K, and 100K, and 90 minutes for 3K.

Chemical compatibility

The polyethersulfone membranes used in the Pierce Protein Concentrators are compatible with most standard aqueous biological samples, buffers, and salts (Table 2). Samples containing high levels of cell membranes, fats, or lipids may reduce performance and result in membrane blockage.

Table 2. Concentrator chemical compatibility*

Acids & Bases	Rating	Organics	Rating	Miscellaneous	Rating
Acetic acid (25%)	A	Acetone	NR	Ammonium sulfate (saturated)	A
Formic acid (5%)	A	Acetonitrile	NR	Glycerine (70%)	A
Hydrochloric acid (1 M)	A	Benzene (100%)	NR	Guanidine HCL (6 M)	A
Lactic acid (5%)	A	Chloroform (1%)	NR	Imidazole (300 mM)	A
Nitric acid (10%)	A	Dimethyl sulfoxide (5%)	A	Phosphate buffer (1 M)	A
Sodium hydroxide (2.5 M)	NR	Ethanol (70%)	A	Polyethylene glycol (10%)	A
Sulfamic acid (5%)	A	Ethyl acetate (100%)	NR	Sodium carbonate (20%)	A
Trifluoroacetic acid (10%)	A	Formaldehyde (30%)	A	Sodium deoxycholate (5%)	A
		Hydrocarbons (aromatic)	NR	Sodium dodecylsulfate (0.1 M)	A
		Hydrocarbons (chlorinated)	NR	Sodium hypochlorite (200 ppm)	A
		Isopropanol (70%)	A	Sodium nitrate (1%)	A
		Mercaptoethanol (1 M)	NR	Tween 20 (0.1%)	A
		Pyridine (100%)	NR	Triton X-100 (0.1%)	A
		Tetrahydrofuran (5%)	NR	Urea (8 M)	A
		Toluene (1%)	NR		

A = Acceptable NR = Not Recommended

*Concentrations listed are provided as guidelines and do not necessarily represent maximum tolerances. Some compatible chemicals might modify the apparent molecular weight of molecules in the sample and/or the molecular weight cutoff rating of the membrane.

Selecting the right format

The Thermo Scientific Pierce Protein Concentrators are easy-to-use centrifugal devices that enable fast processing and excellent recovery of protein samples. These disposable ultrafiltration devices contain a polyethersulfone (PES) membrane for the concentration, desalting, and buffer exchange of biological samples, such as tissue culture media, antiserum, monoclonal antibody preparations, and chromatography fractions. They can also be used to remove unincorporated label following protein modification reactions. The PES membrane in these devices is available in five distinct molecular weight cutoffs (MWCO) of 3K, 5K, 10K, 30K, and 100K, and can be used for processing sample volumes from 100 µL to 100 mL. The MWCOs are etched on the sides of the concentrators for easy identification, while a clear window with graduations on the side of each device allows for visual determination of the concentrated sample (retentate) volume. The unique design provides reliable and consistent results. Multiple device sizes are available to handle maximum sample volumes of 0.5 mL, 6 mL, 20 mL, and 100 mL.

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 fixed-angle or swinging-bucket rotors.

The Pierce concentrators are designed for easy handling and sample processing. The upper chamber is wide enough for convenient sample addition. Following concentration, the sample chamber can be simply detached from the filtrate chamber, and the concentrated protein can be easily removed with a pipette tip. The screw top cap eliminates the need to use parafilm when mixing solutions during buffer exchange. In addition, unlike similar products from other vendors, reverse centrifugation is not required to recover concentrated sample from the device. Selecting the appropriate MWCO will depend on the size of your protein. The PES membrane has been rated for retaining molecules with a molecular weight at least two-fold greater than the MWCO rating of the membrane within the device. Reduced recovery may occur when using a concentrator with molecules smaller than the recommended MWCO. Protein recovery will vary depending on the specific protein in the sample and its starting concentration. To achieve >90% recovery of protein, the minimum protein sample concentration should be 0.05 mg/mL.

Table 3. Thermo Scientific Pierce Protein Concentrators selection guide.

Volume Range	0.1 mL– 0.5 mL	2 mL–6 mL	5 mL–20 mL	20 mL–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 proteins solutions were used for each molecular weight cutoff (MWCO).

Pierce Protein Concentrators, 0.5 mL

The Thermo Scientific Pierce Protein Concentrators, 0.5 mL, are ideal for processing samples between 100 and 500 µL. The 0.5 mL concentrators are available in 3K, 10K, 30K, and 100K MWCO. These devices are compatible with most benchtop microcentrifuges with fixed-angle rotors that accommodate 2.2 mL tubes. Centrifuge at 15,000 x *g* until the desired concentration factor is achieved. Up to a 30-fold concentration can be obtained in as little as 10 minutes with protein solutions of 0.1 mg/mL or higher, but times may vary significantly based on molecular weight cutoff and sample concentration or viscosity. The dead-stop volume is approximately 15 µL. Typical protein recovery is >90%.

Table 4. Processing time and protein recovery using Thermo Scientific Pierce Protein Concentrators, 0.5 mL. Four different protein solutions starting with a 0.5 mL volume were concentrated over 30-fold in a fixed angle rotor at 15,000 x *g* at 25°C, using 3K, 10K, 30K, or 100K MWCO devices.

Membrane MWCO (kDa)	Protein solute	Processing time (minutes)	Protein retentate volume (µL)	Protein retentate recovery (%)
3	Cytochrome C, 12 kDa (0.25 mg/mL)	15	67	100
10	Ovalbumin, 45 kDa (0.25 mg/mL)	3	10	95
30	BSA, 66 kDa (0.25 mg/mL)	3	48	96
100	Thyroglobulin, 660 kDa (0.25 mg/mL)	11	9	100

Pierce Protein Concentrators, 6 mL

The Thermo Scientific Pierce Protein Concentrators, 6 mL, are ideal for processing samples between 2 mL and 6 mL. These 6 mL concentrators are available in 3K, 10K, 30K, and 100K MWCO. These devices fit into a swinging bucket or a fixed angle rotor that accommodates 15 mL conical tubes. Centrifuge at 3,000 to 4,000 x *g* until the desired concentration factor is achieved. Greater than 30-fold concentration can be obtained in as little as 15 minutes with protein solutions of 0.1 mg/mL or higher; however, times may vary significantly based on molecular weight cutoff and sample concentration or viscosity. The dead-stop volume is approximately 30 µL. Typical protein recovery is >90%.

Table 5. Processing time and protein recovery using Thermo Scientific Pierce Protein Concentrators, 6 mL. Four different protein solutions starting with a 6 mL volume were concentrated over 30-fold in a swinging bucket rotor at 4,000 x *g* at 25°C, using 3K, 10K, 30K, or 100K MWCO devices.

Membrane MWCO (kDa)	Protein solute	Processing time (minutes)	Protein retentate volume (µL)	Protein retentate recovery (%)
3	Cytochrome C, 12 kDa (0.25 mg/mL)	90	174	94
10	Ovalbumin, 45 kDa (0.25 mg/mL)	15	91	98
30	BSA, 66 kDa (0.25 mg/mL)	15	51	100
100	Thyroglobulin, 660 kDa (0.25 mg/mL)	15	213	97

Pierce Protein Concentrators, 20 mL

The Thermo Scientific Pierce Protein Concentrators, 20 mL, are ideal for processing samples between 5 mL and 20 mL. These 20 mL concentrators are available in 3K, 10K, 30K, and 100K MWCO. These devices fit into a swinging bucket or a fixed angle rotor that accommodates 50 mL conical tubes. Centrifuge at 3,000 to 5,000 x *g* until the desired concentration factor is achieved. Up to a 30-fold concentration can be obtained in as little as 15 minutes with protein solutions of 0.1 mg/mL or higher; however, times may vary significantly based on molecular weight cutoff and sample concentration or viscosity. The dead-stop volume is approximately 50 µL. Typical protein recovery is >90%.

Table 6. Processing time and protein recovery using Thermo Scientific Pierce Protein Concentrators, 20 mL. Four different protein solutions starting with a 20 mL volume were concentrated over 30-fold in a swinging bucket rotor at 4,700 x *g* at 25°C, using 3K, 10K, 30K, or 100K MWCO devices.

Membrane MWCO (kDa)	Protein solute	Processing time (minutes)	Protein retentate volume (µL)	Protein retentate recovery (%)
3	Cytochrome C, 12 kDa (0.25 mg/mL)	60	121	100
10	Ovalbumin, 45 kDa (0.25 mg/mL)	15	528	94
30	BSA, 66 kDa (0.25 mg/mL)	15	216	100
100	Thyroglobulin, 660 kDa (0.25 mg/mL)	15	777	98

Pierce Protein Concentrators, 100 mL

The Thermo Scientific Pierce Protein Concentrators, 100 mL, are ideal for processing samples between 20 mL and 100 mL. These 100 mL concentrators are available in 5K, 10K, 30K, and 100K MWCO. The devices can be used directly for volumes under 90 mL; when adding volumes between 90 mL and 100 mL, it is recommended to parafilm the cap to the bottle to help prevent leakage. These devices fit into a swinging bucket or a fixed angle rotor that accommodates 250 mL bottles. Centrifuge at 1,200 x *g* until the desired concentration factor is achieved. Up to a 30-fold concentration can be obtained in as little as 15 minutes with protein solutions of 0.1 mg/mL or higher; however, times may vary significantly based on molecular weight cutoff and sample concentration or viscosity. The dead-stop volume is approximately 350 µL. Typical protein recovery is >90%.

Table 7. Processing time and protein recovery using Thermo Scientific Pierce Protein Concentrators, 100 mL. Four different protein solutions starting with a 90 mL volume were concentrated over 30-fold in a swinging bucket rotor at 1,200 x *g* at 25°C, using 5K, 10K, 30K, or 100K MWCO devices.

Membrane MWCO (kDa)	Protein solute	Processing time (minutes)	Protein retentate volume (mL)	Protein retentate recovery (%)
3	Cytochrome C, 12 kDa (0.25 mg/mL)	90	1.9	98
10	Ovalbumin, 45 kDa (0.25 mg/mL)	15	3.5	96
30	BSA, 66 kDa (0.25 mg/mL)	15	2.8	95
100	Thyroglobulin, 660 kDa (0.25 mg/mL)	15	2.5	92

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

Endotoxin removal using affinity chromatography

Overview

Biotechnology refers to biological processes that have been engineered. Following the development of recombinant DNA technology, peptides, hormones, and proteins that were originally extracted from tissues and secretions can now be produced synthetically with high purity and yield. Protein-based engineering is now one of the fastest growing areas in research.

Endotoxin contamination is a common problem with recombinant proteins purified from gram-negative bacteria such as *E. coli.* Endotoxins are heat-stable molecules associated with the outer membranes of certain gram-negative bacteria. When these bacterial cells die, their cell membranes rupture and endotoxins (which are essential lipopolysacchride (LPS) components of the cell walls) are released into the surrounding environment. Endotoxins are frequent contaminants of protein solutions derived from bioproduction. Endotoxins are also toxic to cells grown in tissue culture. Traditional endotoxin removal methods and their limitations are described in Table 1.

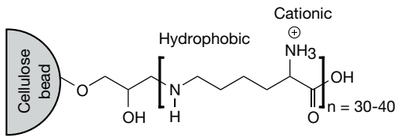
Table 1. Benefits of Thermo Scientific™ Pierce™ High Capacity Endotoxin Removal Resin over traditional methods.

Traditional endotoxin removal method	Limitations	Benefit from Pierce High Capacity Endotoxin Removal Resin
Anion-exchange chromatography	Loss of negatively charged protein	Successfully process proteins across a range of isoelectric points
Ultrafiltration	Only removes large endotoxin aggregates, so it is compatible only with low molecular weight proteins. Endotoxin bound to toxin will not be effectively removed. Technique also exerts strong physical forces on the protein	Successfully process proteins ranging in molecular weight from 12 to 150 kDa
Membrane-based chromatography	Reduced endotoxin binding capacity compared to resin based methods; non-reusable	Resin binds up to 2,000,000 EU/mL† and can be reused up to 10 times with no loss in performance
Polymyxin B affinity ligand	Ligand exhibits neurotoxicity and sodium deoxycholate buffers cause renal tubular necrosis	Poly(ε-lysine) is a safe, nontoxic polymer of the natural amino acid lysine and is commonly used as a food preservative

† One endotoxin unit/mL (EU/mL) equals approximately 0.1 ng endotoxin/mL of solution.

Products designed for efficient endotoxin removal

Ultrafiltration, Polymixin B affinity resin, or resin- or membrane-based chromatography are the traditional methods for endotoxin removal. All have limitations in protein recovery, endotoxin binding capacity, and/or have toxicity concerns. Thermo Fisher Scientific offers a modified ε-poly-L-lysine [poly(ε-lysine)] affinity resin, which is a safe, nontoxic polymer of the natural amino acid lysine that is commonly used as a food preservative (Figure 1). This resin is available as a slurry to pack a custom column, or in convenient pre-packed, single-use spin columns optimized for different sample volumes.



Poly(ε-lysine) resin
High Capacity Endotoxin Removal Resin

Figure 1. The poly(ε-lysine) affinity ligand binds endotoxins through both ionic and hydrophobic interactions. The multiple ε-aminobutyl groups impart both a positive charge via the primary amines as well as a hydrophobic characteristic via the butyl spacer between primary amines. The hydrophilic nature of the porous cellulose base–matrix is masked by thorough derivatization of its interior and exterior surfaces with the poly(ε-lysine) ligand.

Tools to quantitate endotoxin removal

It is critical to measure endotoxin levels in protein solutions. In the early 1950s Frederick Bang discovered that the horseshoe crab's blood cells (amoebocytes) contain a clotting agent that attaches to dangerous *endotoxins* produced by gram negative bacteria. The Limulus Amebocyte Lysate (LAL) endotoxin test gel-clot method was developed to measure endotoxin levels (Figure 2), and was approved by the United States Food and Drug Administration (FDA) in 1983. This method can be used to determine if products or materials are *"endotoxin free".*

As biotechnology has evolved, methods have been developed to successfully reduce the contamination of unwanted pathogens and increase the production volumes of these endotoxin-free proteins.

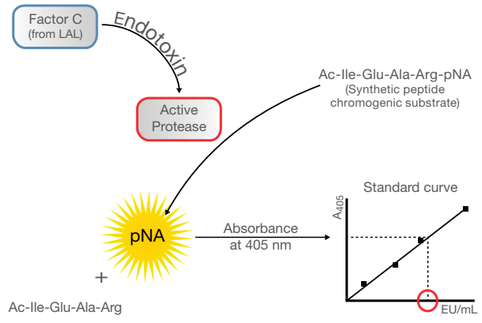


Figure 2. LAL endotoxin quantitation assay overview.

Resin performance data

Binding capacity

Table 2. Efficiently remove endotoxins from heavily contaminated samples. Thermo Scientific™ Pierce™ High Capacity Endotoxin Removal Spin Columns, 0.5 mL, were challenged for 1 hour with protein (2 mL of 1 mg/mL BSA) containing 5,000 to 500,000 EU/mL from *E. coli* strain 055:B5. Even at the highest endotoxin levels clean-up was >98%.

Initial endotoxin concentration (EU/mL)*	Final endotoxin concentration (EU/mL)	Endotoxin removal efficiency	Protein recovery
5,000	<1	99.98%	>90%
12,500	<1	99.99%	>90%
25,000	1.26	99.99%	>90%
50,000	7.1	99.99%	>90%
250,000	32	99.98%	>90%
500,000	9,600	98.08%	>90%

*One endotoxin unit/mL (EU/mL) equals approximately 0.1 ng endotoxin/mL of solution.

Protein compatibility

Table 3. Clean-up proteins of various molecular weights and isoelectric points with minimal sample. Pierce High Capacity Endotoxin Removal Spin Columns, 0.5 mL, were challenged for 1 hour with different proteins of various molecular weight and charge. In all samples endotoxin removal was >99% and protein recovery was >85%.

Protein (1 mg/mL)	Molecular weight (Da)	Isoelectric point (pI)	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)	Endotoxin removal efficiency	Protein recovery
Cytochrome C	12,000	10.6	10,000	1.35	>99.9%	86%
Myoglobin	17,000	6.8	10,000	3.67	>99.9%	87%
Bovine serum albumin (BSA)	66,000	4.9	10,000	0.80	>99.9%	85%
Bovine gamma globulin (BGG)	150,000	7.4	10,000	4.60	>99.9%	92%

Endotoxin removal compared to other suppliers

Table 4. Thermo Scientific Pierce High Capacity Endotoxin Removal Resin delivers the best sample clean-up. Various endotoxin removal resins were challenged with 2 mL of 1 mg/mL BSA containing 25,000 EU/mL according to the respective manufacturers' procedures. The poly(ε-lysine)-based Pierce High Capacity Endotoxin Removal Resin consistently exhibited the highest endotoxin removal efficiency and the highest protein recovery.

	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)	Protein recovery
Thermo Scientific Pierce High Capacity Endotoxin Removal Resin	**25,000**	**<1**	**91%**
Thermo Scientific Detoxi-Gel Endotoxin Removal Gel [Thermo Fisher Scientific]	25,000	77	74%
Affi-Prep Polymyxin Matrix [Bio-Rad]	25,000	13	88%
Polymyxin B-Agarose [Sigma]	25,000	900	63%
ReductEtox [Sterogene]	25,000	13,900	92%
EndoTrap red [Hyglos]	25,000	64	82%

Sample compatibility

Table 5. Thermo Scientific Pierce High Capacity Endotoxin Removal Resin removes endotoxins from various strains of *E. coli.* Pierce High Capacity Endotoxin Removal Spin Columns, 0.5 mL, were challenged with 1 mL BSA (1 mg/mL) spiked with 10,000 EU from different strains of *E. coli.* In all samples removal was performed with >99% efficiency.

E. coli strain	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)	Endotoxin removal efficiency	Protein recovery
011:B4	10,000	0.36	>99.9%	83%
026:B6	10,000	4.18	>99.9%	88%
0127:B8	10,000	0.87	>99.9%	87%
0128:B12	10,000	5.01	>99.9%	88%

Table 6. Process protein and antibody samples from a variety of sources. Protein samples from cell culture supernatants, *E. coli* lysates or human serum were processed with the Pierce High Capacity Endotoxin Removal Spin Columns. In all samples endotoxin levels were reduced to less than 1 EU/mL.

Protein source	Initial endotoxin concentration (EU/mL)	Final endotoxin concentration (EU/mL)
Anti-Fractalkine from cell culture supernatant	8.26	<1
His-tag GFP from *E. coli*	9,780	<1
IgG from human serum	78	<1

Selecting the right format

Thermo Scientific Pierce High Capacity Endotoxin Removal Resin selectively binds and removes endotoxins from protein, peptide and antibody samples using a modified ε-poly-L-lysine [poly(ε-lysine)] affinity ligand. Endotoxin levels in biological samples are reduced by up to 99% in as fast as 1 hour using our spin column format, and protein recovery is ≥ 85%. Pierce High Capacity Endotoxin Removal Resin is available as a slurry to pack a custom column or in convenient pre-packed, single-use spin columns optimized for different sample volumes.

Highlights:

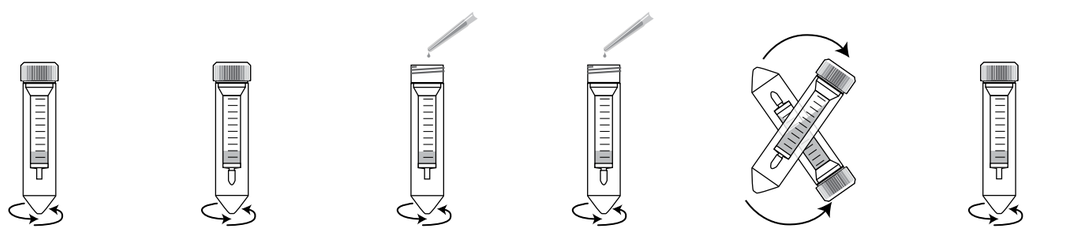
- **High capacity**—bind up to 2,000,000 EU/mL to eliminate >99% of endotoxins
- **Durable**—reuse resin up to 10 times
- **Selective**—recover ≥85% of your protein sample
- **High performance**—complies with FDA guidelines by reducing final EU concentration to <5 EU/mL
- **Fast**—our spin column format enables endotoxin depletion typically within 1 hour
- **Clean**—single-use spin columns avoid cross contamination of samples
- **Optimized**—spin columns are optimized for different sample volumes
- **Economical**—large-volume discounts available

Table 7. Thermo Scientific endotoxin removal products selection guide by format.

Format:	Spin column	Spin column	Spin column	Loose resin
				
Sample volume processing	0.5–1 mL	1–4 mL	2–10 mL	Varies
Supplied as	25% slurry in 20% ethanol	25% slurry in 20% ethanol	25% slurry in 20% ethanol	50% slurry in 20% ethanol
Packaging options	5 or 25 columns/pack	5 or 25 columns/pack	5 or 25 columns/pack	10, 100 or 250 mL bottles

Spin columns

The pre-packed spin column format is a fast, single-use method to remove 99% of endotoxins from protein samples in as fast as 1 hour (Figure 3). These spin columns use a batch format to bind and remove endotoxins while allowing for >85% protein recovery. Three pre-packed spin-column sizes are available to process protein samples of different volumes.



1. Centrifuge at 500 x *g* for 1 minute to remove the storage buffer.
2. Regenerate with 0.2 N NaOH (overnight) or 0.2 N NaOH in 95% ethanol for 1 to 2 hours.
3. Wash with 2 M NaCl followed by ET-free water.
4. Equilibrate with ET-free buffer, pH 6–8. Repeat three times.
5. Add sample and incubate at 4°–22°C with gentle end-over-end mixing for 1 hour.
6. Centrifuge at 500 x *g* for 1 minute to collect the sample.

Figure 3. Protocol for Pierce High Capacity Endotoxin Removal Spin Columns.

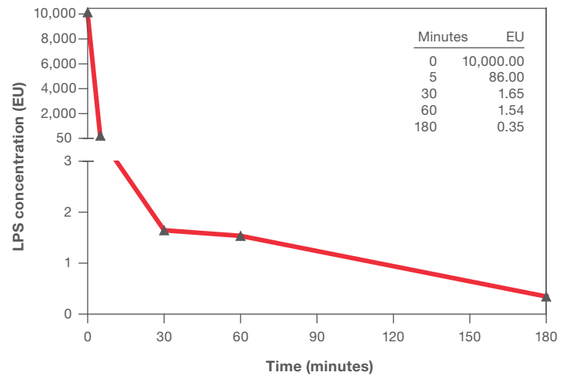


Figure 4. Remove endotoxins from protein samples in 1 hour. Pierce High Capacity Endotoxin Removal Spin Columns, 0.5 mL, were challenged for different time intervals with 1 mL samples containing 1 mg/mL BSA spiked with 10,000 EU. The final endotoxin concentration was determined using the Thermo Scientific™ Pierce™ LAL Chromogenic Endotoxin Quantitation Kit (Cat. No. PI88282). A one-hour incubation time provided 99% endotoxin removal, and longer incubation periods accomplished even greater endotoxin removal.

Loose resin

Pierce High Capacity Endotoxin Removal Resin is available as a slurry in 10, 100 or 250 mL pack sizes for custom packing of endotoxin removal columns, and can be used with gravity flow systems or automated chromatography systems with flow rates ranging from 10 to 15 mL/hour.

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

Endotoxin quantitation kit

The Thermo Scientific Pierce LAL Chromogenic Endotoxin Quantitation Kit measures the amount of endotoxin in a protein, peptide or antibody sample using the LAL assay. Designed for up to 50 samples, this quantitation kit is ideal for processing a few samples at a time compared to other high-throughput quantitation kits. The endotoxin concentration in a sample is measured via a chromogenic signal generated in the presence of endotoxins. Samples can be measured on a microplate absorbance reader at 405 nm. A standard curve is created using the *E. coli* endotoxin standard included with each kit to calculate endotoxin levels as low as 0.1 EU/mL (Figure 4). Protein and antibody samples can be assayed in as few as 30 minutes.

Highlights:

- **Sensitive**—detect as little as 0.1 EU/mL
- **Fast**—perform this assay in 30 minutes
- **Economical**—assay requires only 10 µL of a protein sample
- **Accurate**—*E. coli* O111:B4 standard in each kit enables accurate endotoxin quantitation
- **Versatile**—405 nm absorbance reading is compatible with common ELISA plate readers

Ordering information

Product	Quantity	Cat. No.
2K MWCO membrane products		
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	50 devices	PI69580
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	250 devices	PI69553
Slide-A-Lyzer G2 Dialysis Cassettes, 30 mL	6 cassettes	PI87720
Slide-A-Lyzer G2 Dialysis Cassettes, 70 mL	6 cassettes	PI87721
Slide-A-Lyzer G2 Dialysis Cassettes, 15 mL	8 cassettes	PI87719
Slide-A-Lyzer G2 Dialysis Cassettes, 0.25–0.75 mL	10 cassettes	PI87717
Slide-A-Lyzer G2 Dialysis Cassettes, 1–3 mL	10 cassettes	PI87718
Slide-A-Lyzer Dialysis Cassettes, 12–30 mL	6 cassettes	PI66230
Slide-A-Lyzer Dialysis Cassettes, 3–12 mL	8 cassettes	PI66212
Slide-A-Lyzer Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI66205
Slide-A-Lyzer Dialysis Cassettes, 0.5–3 mL	10 cassettes	PI66203
Slide-A-Lyzer Dialysis Flasks, 250 mL	4 flasks	PI87760
3.5K MWCO membrane products		
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL and Float *Sufficient caps are included*	Kit/ 10 devices	PI69558
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	50 devices	PI69550
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	250 devices	PI69552
Slide-A-Lyzer MINI Dialysis Devices, 0.5 mL	25 devices	PI88400
Slide-A-Lyzer MINI Dialysis Devices, 2 mL	25 devices	PI88403
Slide-A-Lyzer G2 Dialysis Cassettes, 70 mL	6 cassettes	PI87726
Slide-A-Lyzer G2 Dialysis Cassettes, 30 mL	6 cassettes	PI87725
Slide-A-Lyzer G2 Dialysis Cassettes, 15 mL	8 cassettes	PI87724
Slide-A-Lyzer G2 Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI87722
Slide-A-Lyzer G2 Dialysis Cassettes, 1–3 mL	10 cassettes	PI87723
Slide-A-Lyzer Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI66333
Slide-A-Lyzer Dialysis Cassette Kit, 0.1–0.5 mL Contains 10 cassettes, 10 buoys and 10 syringes	Kit	PI66335
Slide-A-Lyzer Dialysis Cassettes, 0.5–3 mL	10 cassettes	PI66330
Slide-A-Lyzer Dialysis Cassette Kit, 0.5–3 mL Contains 10 cassettes, 10 buoys and 10 syringes	Kit	PI66332
Slide-A-Lyzer Dialysis Cassettes, 12–30 mL	6 cassettes	PI66130
Slide-A-Lyzer Dialysis Cassettes, 3–12 mL	8 cassettes	PI66110
Slide-A-Lyzer Dialysis Cassette Kit, 3–12 mL	Kit	PI66107
Slide-A-Lyzer Dialysis Flasks, 230 mL	4 flasks	PI87761
SnakeSkin Dialysis Tubing, 16 mm *Sufficient for: Approx. 10 mL per 5 cm length plus 2.5 cm at each end for closure) yields 100 uses*	35 ft/pkg	PI88242
SnakeSkin Dialysis Tubing, 22 mm *Sufficient for: Approx. 19 mL per 5 cm length (plus 2.5 cm at each end for closure) yields 100 uses*	35 ft/pkg	PI68035
SnakeSkin Dialysis Tubing, 35 mm *Sufficient for: Approx. 48 mL per 5 cm length (plus 2.5 cm at each end for closure) yields 100 uses*	35 ft/pkg	PI88244
Pierce 96-well Microdialysis Plate Formulation: Dialysis membrane framed in plastic insert with 96-well deep-well plate *Sufficient for: Dialysis of 96 samples 10 to 100 µL each* Kit contents: 8-microdialysis device strip, 12 strips, 96-well deep-well plate, 1 plate	1-plate set	PI88262

Product	Quantity	Cat. No.
3.5K MWCO membrane products (continued)		
96-well Deep-well Plate, 2.2 mL Formulation: Polypropylene deep-well assay plate *Sufficient for: 96 microdialysis devices*	1 plate	PI88261
Pierce Plate Seal for Pierce 96-well Microdialysis Plates Formulation: Adhesive coated plastic sheet, perforated to 8-well strips fitting microdialysis plate *Sufficient for: 12 x 8 microdialysis plate wells*	1 sheet	PI88269
7K MWCO membrane products		
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	50 devices	PI69560
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	250 devices	PI69562
Slide-A-Lyzer G2 Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI87727
Slide-A-Lyzer G2 Dialysis Cassettes, 1–3 mL	10 cassettes	PI87728
Slide-A-Lyzer Dialysis Cassettes, 3–12 mL	8 cassettes	PI66710
Slide-A-Lyzer Dialysis Cassette Kit, 3–12 mL Contains 8 cassettes, 8 buoys and 10 syringes	Kit	PI66707
Slide-A-Lyzer Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI66373
Slide-A-Lyzer Dialysis Cassettes, 0.5–3 mL	10 cassettes	PI66370
Slide-A-Lyzer Dialysis Cassette Kit, 0.5–3 mL Contains 10 cassettes, 10 buoys and 10 syringes	Kit	PI66372
SnakeSkin Dialysis Tubing, 22 mm *Sufficient for: Approx. 19 mL per 5 cm length (plus 2.5 cm at each end for closure) yields 100 uses*	35 ft/pkg	PI68700
10K MWCO membrane products		
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL Plus Microtubules *Sufficient caps are included*	10 devices	PI69574
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	50 devices	PI69570
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL *Sufficient caps are included*	250 devices	PI69572
Slide-A-Lyzer MINI Dialysis Devices, 10–100 µL Plus Float *Sufficient caps are included*	Kit/ 10 devices	PI69576
Slide-A-Lyzer MINI Dialysis Devices, 0.5 mL	25 devices	PI88401
Slide-A-Lyzer MINI Dialysis Devices, 2 mL	25 devices	PI88404
Slide-A-Lyzer G2 Dialysis Cassettes, 30 mL	6 cassettes	PI87732
Slide-A-Lyzer G2 Dialysis Cassettes, 70 mL	6 cassettes	PI87733
Slide-A-Lyzer G2 Dialysis Cassettes, 15 mL	8 cassettes	PI87731
Slide-A-Lyzer G2 Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI87729
Slide-A-Lyzer G2 Dialysis Cassettes, 1–3 mL	10 cassettes	PI87730
Slide-A-Lyzer Dialysis Cassettes, 12–30 mL	6 cassettes	PI66830
Slide-A-Lyzer Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI66383
Slide-A-Lyzer Dialysis Cassettes, 0.1–0.5 mL	50 cassettes	PI66384
Slide-A-Lyzer Dialysis Cassette Kit, 0.1–0.5 mL Contains 10 cassettes, 10 buoys and 10 syringes	Kit	PI66385
Slide-A-Lyzer Dialysis Cassettes, 0.5–3 mL	10 cassettes	PI66380
Slide-A-Lyzer Dialysis Cassettes, 0.5–3 mL	50 cassettes	PI66381
Slide-A-Lyzer Dialysis Cassette Kit, 0.5–3 mL Contains 10 cassettes, 10 buoys and 10 syringes	Kit	PI66382
Slide-A-Lyzer Dialysis Cassettes, 3–12 mL	8 cassettes	PI66810
Slide-A-Lyzer Dialysis Cassettes, 3–12 mL	50 cassettes	PI66811
Slide-A-Lyzer Dialysis Cassette Kit, 3–12 mL Contains 8 cassettes, 8 buoys and 10 syringes	Kit	PI66807

Ordering information

Product	Quantity	Cat. No.
10K MWCO membrane products (continued)		
Slide-A-Lyzer Dialysis Flask, 250 mL	4 flasks	PI87762
SnakeSkin Dialysis Tubing, 22 mm *Sufficient for: Approx. 19 mL per 5 cm length (plus 2.5 cm at each end for closure) yields 100 uses*	35 ft./pkg	PI68100
SnakeSkin Dialysis Tubing, 16 mm *Sufficient for: Approx. 10 mL per 5 cm length (plus 2.5 cm at each end for closure) yields 100 uses*	35 ft./pkg.	PI88243
SnakeSkin Dialysis Tubing, 35 mm *Sufficient for: Approx. 48 mL per 5 cm length (plus 2.5 cm at each end for closure) yields 100 uses*	35 ft./pkg.	PI88245
Pierce 96-well Microdialysis Plate, Formulation: Dialysis membrane framed in plastic insert with 96-well deep-well plate *Sufficient for dialysis of 96 samples 10 to 100 µL each* Kit contents: 8-microdialysis device strip, 12 strips, 96-well deep-well plate, 1 plate	1-plate set	PI88260
96-well Deep-well Plate, 2.2 mL Formulation: Polypropylene deep-well assay plate *Sufficient for 96 microdialysis devices*	1 plate	PI88261
Pierce Plate Seal for Pierce 96-well Microdialysis Plates Formulation: Adhesive coated plastic sheet, perforated to 8-well strips fitting microdialysis plate *Sufficient for: 12 x 8 microdialysis plate wells*	1 sheet	PI88269
Gamma-irradiated 10K MWCO membrane		
Slide-A-Lyzer Dialysis Cassettes, 3–12 mL	8 cassettes	PI66453
Slide-A-Lyzer Dialysis Cassettes, 12–30 mL	8 cassettes	PI66456
Slide-A-Lyzer Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI66454
Slide-A-Lyzer Dialysis Cassettes, 0.5–3 mL	10 cassettes	PI66455
Slide-A-Lyzer G2 Dialysis Cassettes, 0.5 mL	10 cassettes	PI88250
Slide-A-Lyzer G2 Dialysis Cassettes, 3 mL	10 cassettes	PI88251
Slide-A-Lyzer G2 Dialysis Cassettes, 15 mL	8 cassettes	PI88252
Slide-A-Lyzer G2 Dialysis Cassettes, 30 mL	6 cassettes	PI88253
Slide-A-Lyzer G2 Dialysis Cassettes, 70 mL	6 cassettes	PI88254

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

Product	Quantity	Cat. No.
20K MWCO membrane products		
Slide-A-Lyzer MINI Dialysis Devices, 0.1–0.5 mL	25 devices	PI88402
Slide-A-Lyzer MINI Dialysis Devices, 2 mL	25 devices	PI88405
Slide-A-Lyzer G2 Dialysis Cassettes, 30 mL	6 cassettes	PI87737
Slide-A-Lyzer G2 Dialysis Cassettes, 70 mL	6 cassettes	PI87738
Slide-A-Lyzer G2 Dialysis Cassettes, 15 mL	8 cassettes	PI87736
Slide-A-Lyzer G2 Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI87734
Slide-A-Lyzer G2 Dialysis Cassettes, 0.5–3 mL	10 cassettes	PI87735
Slide-A-Lyzer Dialysis Cassettes, 12–30 mL	6 cassettes	PI66030
Slide-A-Lyzer Dialysis Cassettes, 3–12 mL	8 cassettes	PI66012
Slide-A-Lyzer Dialysis Cassettes, 0.1–0.5 mL	10 cassettes	PI66005
Slide-A-Lyzer Dialysis Cassettes, 0.5–3 mL	10 cassettes	PI66003
Slide-A-Lyzer Dialysis Flasks, 250 mL	4 flasks	PI87763
Product accessories		
Slide-A-Lyzer MINI Dialysis Device Floats *Holds 25 MINI Dialysis Units*	4 floats	PI69588
Slide-A-Lyzer Cassette Float Buoys, Grey *Holds one 3–12 mL cassette*	8 buoys	PI66432
Slide-A-Lyzer Cassette Float Buoys, White *Holds one 0.1–0.5 mL or 0.5–3 mL cassette*	10 buoys	PI66430
Slide-A-Lyzer Cassette Carousel Float Buoy *Holds ten 0.1–0.5 mL or 0.5–3 mL cassettes*	1 buoy	PI66431
Slide-A-Lyzer Cassette Syringes, 1 mL*	10 syringes	PI66494
Slide-A-Lyzer Cassette Syringes, 5 mL*	10 syringes	PI66490
Slide-A-Lyzer Cassette Syringes, 20 mL*	10 syringes	PI66493
Slide-A-Lyzer Dialysis Flotation Disks, for use with Slide-A-Lyzer Dialysis Flasks *Reusable; each disk holds 1 flask*	6 disks	PI87759
SnakeSkin Dialysis Tubing Clips	6 clips	PI68011

*Each syringe comes with 18-gauge 1-inch beveled needles. And can also be used with the new Slide-A-Lyzer G2 Dialysis Cassettes.

Ordering information

Product	Quantity	Cat. No.
Desalting products		
Zeba Micro Spin Desalting Columns, 7K MWCO, 75 µL *Sufficient for 25 samples, each 2–12 µL*	25 columns	PI89877
Zeba Micro Spin Desalting Columns, 7K MWCO, 75 µL *Sufficient for 50 samples, each 2–12 µL*	50 columns	PI89878
Zeba Spin Desalting Columns, 7K MWCO, 0.5 mL *Sufficient for 25 samples, each 30–130 µL*	25 columns	PI89882
Zeba Spin Desalting Columns, 7K MWCO, 0.5 mL *Sufficient for 50 samples, each 30–130 µL*	50 columns	PI89883
Zeba Spin Desalting Columns, 7K MWCO, 2 mL *Sufficient for 5 samples, each 200–700 µL*	5 columns	PI89889
Zeba Spin Desalting Columns, 7K MWCO, 2 mL *Sufficient for 25 samples, each 200–700 µL*	25 columns	PI89890
Zeba Spin Desalting Columns, 7K MWCO, 5 mL *Sufficient for 5 samples, each 500–2,000 µL*	5 columns	PI89891
Zeba Spin Desalting Columns, 7K MWCO, 5 mL *Sufficient for 25 samples, each 500–2,000 µL*	25 columns	PI89892
Zeba Spin Desalting Columns, 7K MWCO, 10 mL *Sufficient for 5 samples, each 700 µL–4 mL*	5 columns	PI89893
Zeba Spin Desalting Columns, 7K MWCO, 10 mL *Sufficient for 25 samples, each 700 µL–4 mL*	25 columns	PI89894
Zeba Spin Desalting Plates, 7K MWCO *Sufficient for 2 × 96 samples, each 20–100 µL*	2 plates	PI89807
Zeba Spin Desalting Plates, 7K MWCO *Sufficient for 4 × 96 samples, each 20–100 µL*	4 plates	PI89808
Zeba Desalting Chromatography Cartridges, 7K MWCO, 1 mL *Sufficient for samples requiring 1 mL of resin for separation*	5 cartridges	PI89934
Zeba Desalting Chromatography Cartridges, 7K MWCO, 5 mL *Sufficient for samples requiring 5 mL of resin for separation*	5 cartridges	PI89935
Zeba Micro Spin Desalting Columns, 40K MWCO, 75 µL *Sufficient for 25 samples, each 5–14 µL*	25 columns	PI87764
Zeba Micro Spin Desalting Columns, 40K MWCO, 75 µL *Sufficient for 50 samples, each 5–14 µL*	50 columns	PI87765
Zeba Spin Desalting Columns, 40K MWCO, 0.5 mL *Sufficient for 25 samples, each 70–200 µL*	25 columns	PI87766

Product	Quantity	Cat. No.
Desalting products (continued)		
Zeba Spin Desalting Columns, 40K MWCO, 0.5 mL *Sufficient for 50 samples, each 70–200 µL*	50 columns	PI87767
Zeba Spin Desalting Columns, 40K MWCO, 2 mL *Sufficient for 5 samples, each 200–900 µL*	5 columns	PI87768
Zeba Spin Desalting Columns, 40K MWCO, 2 mL *Sufficient for 25 samples, each 200–900 µL*	25 columns	PI87769
Zeba Spin Desalting Columns, 40K MWCO, 5 mL *Sufficient for 5 samples, each 300–2,000 µL*	5 columns	PI87770
Zeba Spin Desalting Columns, 40K MWCO, 5 mL *Sufficient for 25 samples, each 300–2,000 µL*	25 columns	PI87771
Zeba Spin Desalting Columns, 40K MWCO, 10 mL *Sufficient for 5 samples, each 1–4 mL*	5 columns	PI87772
Zeba Spin Desalting Columns, 40K MWCO, 10 mL *Sufficient for 25 samples, each 1–4 mL*	25 columns	PI87773
Zeba 96-well Spin Desalting Plates, 40K MWCO *Sufficient for 2 × 96 samples, each 20–100 µL*	2 plates	PI87774
Zeba 96-well Spin Desalting Plates, 40K MWCO *Sufficient for 4 × 96 samples, each 20–100 µL*	4 plates	PI87775
Related desalting products		
Dextran Desalting Columns, 5K MWCO, 5 mL *Sufficient for 5 samples, each 0.25–1.5 mL*	5 columns	PI43230
Dextran Desalting Columns, 5K MWCO, 10 mL *Sufficient for 5 samples, each 0.5–3 mL*	5 columns	PI43233
Polyacrylamide Spin Desalting Columns, 7K MWCO, 0.7 mL *Sufficient for 25 samples, each 30–120 µL*	25 columns	PI89849
Polyacrylamide Spin Desalting Columns, 7K MWCO, 0.7 mL *Sufficient for 50 samples, each 30–120 µL*	50 columns	PI89862
Polyacrylamide Desalting Columns, 1.8K MWCO, 5 mL *Sufficient for 5 samples, each 0.25–1.25 mL*	5 columns	PI43426
Polyacrylamide Desalting Columns, 6K MWCO, 10 mL *Sufficient for 5 samples, each 0.5–2.5 mL*	5 columns	PI43243

Ordering information

Product	Quantity	Cat. No.
Empty spin column products		
Pierce Micro-Spin Columns Polyethylene filter (30 µm pore size); screw caps with O-rings; press-in bottom plugs *Sufficient for microcentrifuge procedures with 5–100 µL resin*	50 columns	PI89879
Pierce Centrifuge Columns, 0.8 mL Polyethylene filter (30 µm pore size); screw-top caps *Sufficient for microcentrifuge procedures with 40–400 µL resin beds*	50 columns	PI89868
Pierce Centrifuge Columns, 0.8mL Polyethylene filter (30 µm pore size); screw-top caps *Sufficient for microcentrifuge procedures with 40–400 µL resin beds*	4 x 50 columns	PI89869
Pierce Centrifuge Columns, 2 mL Polyethylene filter (30 µm pore size); screw-top and press-on bottom caps *Sufficient for centrifuge procedures with 2 mL resin beds and 15 mL conical centrifuge tubes for collection*	25 columns	PI89896
Pierce Centrifuge Columns, 5 mL Polyethylene filter (30 µm pore size); screw-top and press-on bottom caps *Sufficient for centrifuge procedures with 5 mL resin beds and 15 mL conical centrifuge tubes for collection*	25 columns	PI89897
Pierce Centrifuge Columns, 10 mL Polyethylene filter (30 µm pore size); screw-top and press-on bottom caps *Sufficient for centrifuge procedures with 10 mL resin beds and 50 mL conical centrifuge tubes for collection*	25 columns	PI89898
Pierce Column Extenders Cat. Nos. 89896, 89897, 89898 *Sufficient for increasing column reservoir volumes by approx. 35 mL*	10 extenders	PI69707

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Product	Quantity	Cat. No.
Detergent removal products		
HiPPR Detergent Removal Spin Column Kit, 5 mL	Kit	PI88305
HiPPR Detergent Removal Spin Columns, 0.1 mL	24 columns	PI88306
HiPPR Detergent Removal 96-well Spin (filter) Plates, 0.1 mL	2 plates	PI88307
Pierce Plates Detergent Removal 96-well Spin Plates	2 plates	PI88304
Pierce Detergent Removal Spin Columns, 125 µL	25 columns	PI87776
Pierce Detergent Removal Spin Columns, 0.5 mL	25 columns	PI87777
Pierce Detergent Removal Spin Columns, 2 mL	5 columns	PI87778
Pierce Detergent Removal Spin Columns, 4 mL	5 columns	PI87779
Pierce Detergent Removal Resin	10 mL	PI87780

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Ordering information

Product	Quantity	Cat. No.
Protein concentration products		
Pierce Protein Concentrators PES, 3K MWCO, 0.5 mL	25 units	PI88512
Pierce Protein Concentrators PES, 3K MWCO, 2-6 mL	10 units	PI88514
Pierce Protein Concentrators PES, 3K MWCO, 2-6 mL	24 units	PI88515
Pierce Protein Concentrators PES, 3K MWCO, 5-20 mL	10 units	PI88525
Pierce Protein Concentrators PES, 3K MWCO, 5-20 mL	24 units	PI88526
Pierce Protein Concentrators PES, 5K MWCO, 20-100 mL	4 units	PI88534
Pierce Protein Concentrators PES, 10K MWCO 0.5 mL	25 units	PI88513
Pierce Protein Concentrators PES, 10K MWCO, 2-6 mL	10 units	PI88516
Pierce Protein Concentrators PES, 10K MWCO, 2-6 mL	24 units	PI88517
Pierce Protein Concentrators PES, 10K MWCO, 5-20 mL	10 units	PI88527
Pierce Protein Concentrators PES, 10K MWCO, 5-20 mL	24 units	PI88528
Pierce Protein Concentrators PES, 10K MWCO, 20-100 mL	4 units	PI88535
Pierce Protein Concentrators PES, 30K MWCO, 0.5 mL	25 units	PI88502
Pierce Protein Concentrators PES, 30K MWCO, 2-6 mL	10 units	PI88521
Pierce Protein Concentrators PES, 30K MWCO, 2-6 mL	24 units	PI88522
Pierce Protein Concentrators PES, 30K MWCO, 5-20 mL	10 units	PI88529
Pierce Protein Concentrators PES, 30K MWCO, 5-20 mL	24 units	PI88531
Pierce Protein Concentrators PES, 30K MWCO, 20–100 mL	4 units	PI88536
Pierce Protein Concentrators PES, 100K MWCO, 0.5 mL	25 units	PI88503
Pierce Protein Concentrators PES, 100K MWCO, 2-6 mL	10 units	PI88523
Pierce Protein Concentrators PES, 100K MWCO, 2-6 mL	24 units	PI88524
Pierce Protein Concentrators PES, 100K MWCO, 5-20 mL	10 units	PI88532
Pierce Protein Concentrators PES, 100K MWCO, 5-20 mL	24 units	PI88533

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Product	Quantity	Cat. No.
Endotoxin removal and quantitation		
Pierce High Capacity Endotoxin Removal Spin Columns, 0.25 mL	5 columns	PI88273
Pierce High Capacity Endotoxin Removal Spin Columns, 0.5 mL	5 columns	PI88274
Pierce High Capacity Endotoxin Removal Spin Columns, 0.5 mL	25 columns	PI88275
Pierce High Capacity Endotoxin Removal Spin Columns, 1 mL	5 columns	PI88276
Pierce High Capacity Endotoxin Removal Spin Columns, 1 mL	25 columns	PI88277
Pierce High Capacity Endotoxin Removal Resin	10 mL	PI88270
Pierce High Capacity Endotoxin Removal Resin	100 mL	PI88271
Pierce High Capacity Endotoxin Removal Resin	250 mL	PI88272
Pierce LAL Chromogenic Endotoxin Quantitation Kit, 50 tests	Kit	PI88282

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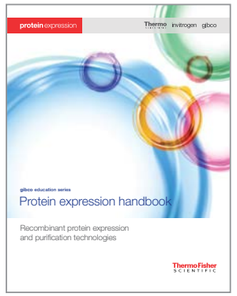

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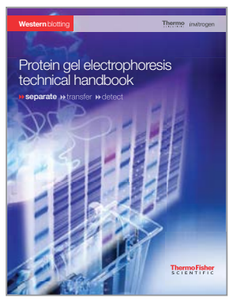

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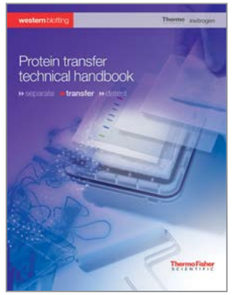

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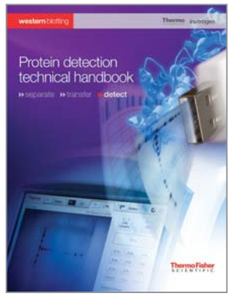

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