ThermoFisher SCIENTIFIC

Sample preparation

Protein clean-up technical handbook

Dialysis • Desalting • Detergent removal • Concentration • Endotoxin removal

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 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 semipermeable membrane to remove small contaminants from protein sample solutions or to exchange buffers. Proteins that are larger than the membrane pore size are retained on one side of the membrane, while smaller molecules diffuse freely through the membrane and approach equilibrium concentrations.

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 proteins travel through the resin faster than the smaller molecules do, 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, and acid or acetone precipitation. However, all these methods can be labor- or time-intensive, or detergent-specific. Proprietary Thermo Scientific™ detergent removal resins enable 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 semipermeable 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.

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

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.

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

Protein dialysis using regenerated cellulose membranes

Overview

Dialysis is a classic technique that facilitates the separation of small unwanted compounds from a solution of macromolecules by selective diffusion. In a typical dialysis application, a sample and a buffer solution are placed on opposite sides of a semipermeable membrane. 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 total volume of buffer (Figure 1).

Through this process, the concentration of small contaminants in the sample can be decreased to acceptable or negligible levels by using a large external buffer volume (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.

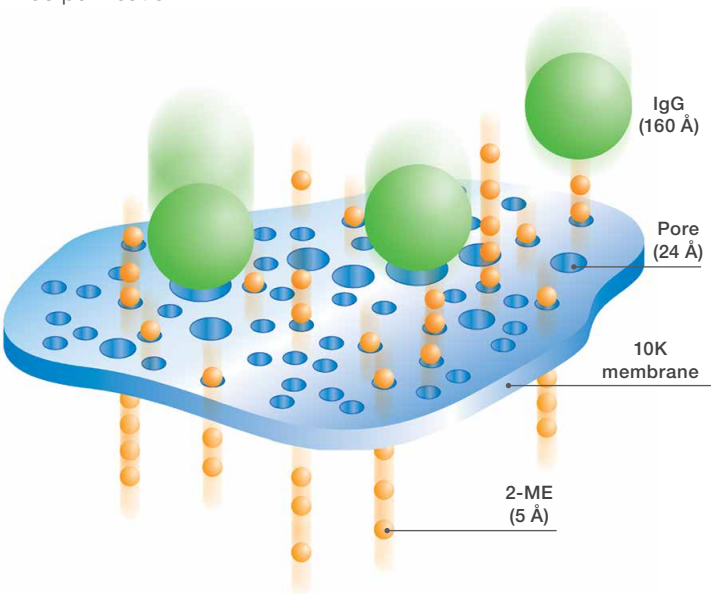


Figure 1. How dialysis membranes work. A dialysis membrane is a semipermeable film (usually a sheet of regenerated cellulose) with pores of various sizes. Molecules larger than the pores cannot pass through the membrane, but small molecules can do so freely. In this manner, dialysis may be used to perform 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 permeability of a membrane is 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 molecules that are retained at >90%, as they cannot pass through 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. MWCO is not a "defined" value, as diffusion of molecules near but below the MWCO will also be significantly slowed. Molecules with molecular weights that is less than 1/10 of the MWCO rating of the membrane will diffuse most rapidly and reliably across the membrane. 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; and the volume, agitation, and frequency of exchange of the external buffer. The rate of dialysis is also directly proportional to the surface area of the membrane relative to the volume of the sample, and the average distance of the sample from the membrane. The more 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.

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 up to 96 samples of 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 they can be separated easily for use as individual dialysis devices.

Each microdialysis device has two regenerated cellulose membranes separated by <2 mm. This combination of short diffusion distance and large surface area enables rapid dialysis. In addition, the small distance between the membranes allows for highly efficient sample recovery using standard laboratory pipettes. The dialysis chambers allow flexibility in the number of units needed per experiment: each device can 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 the 8-unit strips and 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**—for use with sample volumes of 10–100 µL
- **Easy to use**—complete sample loading and retrieval with a standard pipette
- **Flexible**—8-unit detachable 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. The Pierce 96-Well Microdialysis Plate enables the removal of low molecular weight contaminants, buffer exchange, and desalting within 2–4 hours, with typical protein recoveries of >90%.

For more information and protocols, visit thermofisher.com/dialysis

Slide-A-Lyzer MINI Dialysis Devices

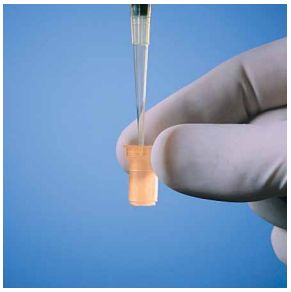
Self-contained devices for sample volumes as small as 10 µL

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 4–8 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 recovery**—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 Dialysis Device and place in tube containing the dialysis buffer; no laborious assembly, device preparation, or expensive equipment 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 2), and are available in MWCOs of 2K, 3.5K, 7K, 10K, and 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 MWCOs. The tubes serve as dialysis reservoirs for easy and self-contained dialysis (Figure 3).



1. Apply sample with a pipette.

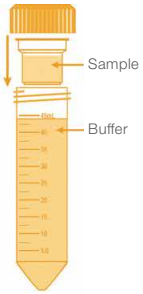
2. Place the Slide-A-Lyzer MINI Dialysis Device into the float.

3. Insert the float into the beaker containing the buffer.

4. Recover sample.

Figure 2. Sample dialysis using a 0.1 mL 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 3. Sample dialysis with a 0.5 mL or 2 mL Slide-A-Lyzer MINI Dialysis Device.

Original 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 the ratio of surface area to sample volume 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 mL 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 and begin dialysis (Figure 4)
- **Fast dialysis**—flat cassette chamber with two membranes provides high ratio of surface area to sample volume, maximizing 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 sample, withdraw excess air, and withdraw the needle.

2. Attach a buoy and dialyze. (Buoys also serve as convenient benchtop 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 4. Slide-A-Lyzer Dialysis Cassette procedure.

Slide-A-Lyzer G3 Dialysis Cassettes

Maximum convenience for high-performance dialysis

Thermo Scientific™ Slide-A-Lyzer™ G3 Dialysis Cassettes are the newest generation of Slide-A-Lyzer cassettes. These third-generation (G3) dialysis products feature a more compact shape, improved self-flotation, less plastic waste, and a secure cap design with wider opening that allows for easier loading and retrieval with a serological pipette (Figure 5). They are available in five volume capacities and in 2K, 3.5K, 10K, and 20K MWCOs.

Highlights:

- **Increased ergonomics**—no more fumbling with floppy dialysis tubing and bags
- **Sample security**—the locking cap secures the sample while still allowing easy sample access
- **Self-flotation**—does not require external flotation device or clips
- **Multiple sizes**—five cassette capacities to optimally match 1–125 mL sample volumes
- **Efficiency**—minimal sample loss
- **Sterile option**—E-beam–irradiated 10K MWCO cassettes are available for applications requiring sterile conditions

The single-use, disposable Slide-A-Lyzer G3 Dialysis Cassettes are available in four MWCOs (2K, 3.5K, 10K, and 20K) and in five different sizes for dialyzing sample volumes between 1 mL and 125 mL. The membrane is composed of low-binding, regenerated cellulose, and the cassettes are manufactured under cleanroom conditions. Selected sizes of 10K MWCO Slide-A-Lyzer G3 Dialysis Cassettes are also available in packages that have been E-beam irradiated to sterilize them. E-beam–irradiated Slide-A-Lyzer G3 Dialysis Cassettes are ideal for workflows involving culturing cells and microorganisms; purifying viruses, DNA, and RNA; or sample preparation for other applications requiring sterile conditions to minimize the risk of sample contamination.

Figure 5. Simple workflow for Slide-A-Lyzer G3 Dialysis Cassettes.

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 MWCOs of 2K, 3.5K, 10K, and 20K, with color coding for easy identification. With Slide-A-Lyzer Dialysis Flasks, typical low molecular weight contaminant removal, buffer exchange, or desalting can be accomplished in as little as 8 hours. The flasks are manufactured under cleanroom conditions. The flasks are constructed with 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 ratio of surface area to sample volume, enabling dialysis of a 250 mL sample in as little as 8 hours
- **High recovery**—flask design maximizes recovery of entire sample volume via opening at top of flask
- **Color-coded frames**—easily identify MWCO by 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 6). A simple screw cap easily and reliably seals the device.

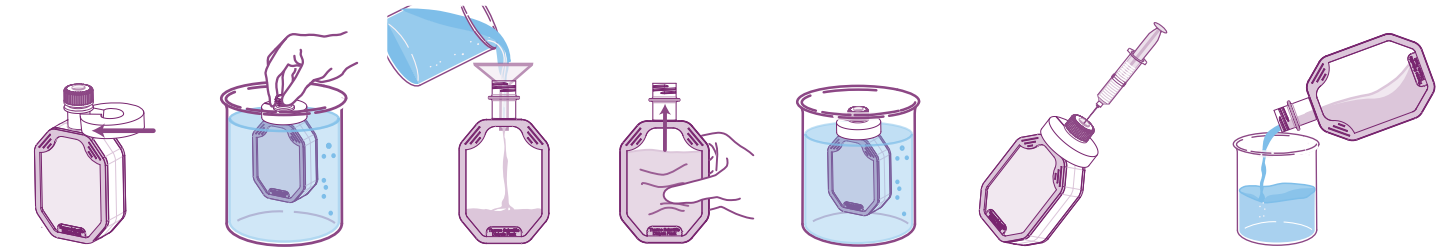


Figure 6. Easy sample loading and recovery with Slide-A-Lyzer Dialysis Flasks.

SnakeSkin Dialysis Tubing

Easier to use than traditional flat tubing

Thermo Scientific™ SnakeSkin™ Dialysis Tubing is a ready-to-use form of traditional dialysis membrane tubing that allows desalting and buffer exchange for 10–100 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 the tubing and close it with a dialysis clip, add the 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 and supplied as an open, pleated (telescoped) tube. It is supplied in 8 inch (20 cm) sticks containing 35 feet of tubing with 16, 22, or 35 mm circular internal diameter (ID) (Table 1). Hydrated SnakeSkin tubing holds ~2–10 mL of sample per centimeter of length. Because SnakeSkin Dialysis Tubing is made from the same type of regenerated cellulose as conventional flat tubing, their dialysis performance is equivalent. SnakeSkin Dialysis Tubing is available with 22 mm ID in three MWCOs: 3.5K, 7K, and 10K. The 3.5K and 10K MWCO membranes are also available with 16 mm and 35 mm ID.

Choose the right dialysis device for your experiments at thermofisher.com/dialysis

Table 1. Dialysis tubing specifications and sample capacity.

Membrane MWCO	Membrane thickness	Tubing diameter	Volume (per cm of tubing)†
3.5K	1.0 mil (25 µm)	16 mm ID	~2.0 mL
7K	1.2 mil (30 µm)	22 mm ID	~3.8 mL
10K	0.9 mil (23 µm)	35 mm ID	~9.6 mL

* Excludes membrane length used for tube closure.

Protein desalting using gel filtration resins

Overview

Thermo Scientific™ Zeba™ Spin Desalting Columns are designed to separate proteins, DNA, and other macromolecules from soluble low molecular weight substances such as salts that may adversely impact the stability of the analytes or interfere with downstream applications. They are widely used for exchanging protein solutions into a more appropriate buffer before subsequent applications such as western blotting, immunoprecipitation, activity assays, and mass spectrometry.

Zeba Desalting Spin Columns yield exceptional desalting and protein-recovery characteristics and provide consistent performance over a wide range of sample sizes and protein concentrations. High protein recovery and greater than 95% contaminant removal are achieved even with dilute protein samples.

Zeba Desalting Spin Columns are highly efficient and have a short protocol. They speed up the process by eliminating the holdup for samples to drop out of the gravity-flow columns, collecting and concentrating cleaned samples in one fraction, requiring no chromatography system, and permitting multisample processing.

- **High performance**—proprietary resin provides 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 elute by gravity flow

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 MWCOs, 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.

Resin performance

Optimized products to enable 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. Spin formats eliminate the need to wait for samples to elute by gravity flow and require no chromatography system, allowing for simultaneous multiple sample processing. 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) 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.

View performance data at **thermofisher.com/desalting**

Available formats

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 2 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. Our broad portfolio of high-performance devices for desalting and buffer exchange provide easy handling, rapid processing, and exceptional recovery for sample volumes of 2–4,000 µL. In addition, two size-exclusion resin options (7K and 40K MWCO) are available. The 7K Zeba desalting resin is recommended for removing <800 Da molecules from macromolecules larger than 7 kDa. The 40K Zeba desalting resin is recommended for removing <1,500 Da molecules from macromolecules larger than 30–40 kDa. Salt removal is typically 95–100%.

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.

Zeba Spin Desalting Plates

The prepacked 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 2).

Table 2. Common specifications of 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 offset	9 mm
Well volume	800 µL
Plate material	Polypropylene
Filter material	Polyethylene
Filter pore size	20 µm
Collection plate maximum volume	150 µL
Maximum centrifuge speed	Up to 1,000 x *g*
Suggested balance plate	Cat. No. 45205

* Plate height includes collection plate for total stack height.

Zeba Desalting Chromatography Cartridges

Thermo Scientific™ Zeba™ Desalting Chromatography Cartridges are available prepacked in 1 mL and 5 mL sizes (Table 3). 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 Cytiva 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 with the 7K MWCO resin.

Choose the right Zeba desalting device at thermofisher.com/desalting

Table 3. Properties of Zeba Desalting Chromatography Cartridges. 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/min (3 mL/min)	1 to 5 mL/min (8 mL/min)
Affinity flow rate (maximum)	0.1 to 1 mL/min (4 mL/min)	0.5 to 2 mL/min (5 mL/min)
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

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 small aggregation numbers, such as 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-intensive, time-intensive, or detergent-specific.

Improved tools to quickly and efficiently remove detergents

Thermo Scientific™ detergent removal products contain a proprietary resin that specifically binds 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.

View protein recovery and application data at **thermofisher.com/detergentremoval**

Available formats

The Thermo Scientific™ detergent removal resins are provided in convenient spin column or plate formats that quickly and efficiently remove ionic, nonionic, and 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 ≤100 µg/mL and >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

Spin columns, loose resins, and kits

The HiPPR Detergent Removal Resin is available in a predispensed 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 prepacked 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.

96-well spin plates

The prepacked 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 25–100 µL of sample per well.

Choose the right detergent removal product for your application 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.

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 (Figure 7). 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.

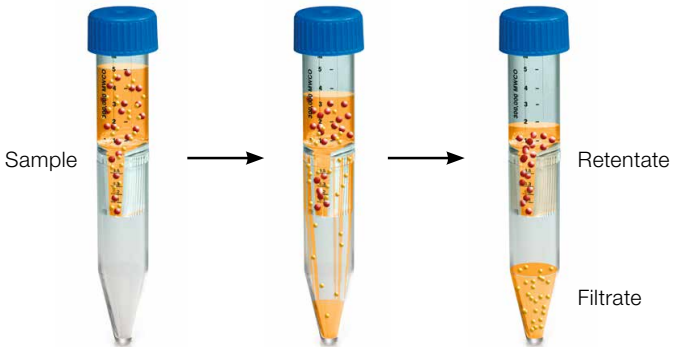


Figure 7. Sample processing using a centrifugal protein concentrator. Images are shown of a concentrator before, during, and after centrifugation.

Membrane types

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 hydrophilic 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 hydrophilic and may show higher protein recovery than other membranes when processing very dilute solutions. Regenerated cellulose is resistant to autoclaving, can be cleaned and reused, and has extended chemical resistance. CTA membranes have high hydrophilicity and very low nonspecific binding. 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.

Available formats

Highlights:

- **Rapid processing**—unique design minimizes membrane fouling, and 10- to 30-fold sample concentration can be achieved in 5–30 minutes for the 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

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, or 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 MWCOs 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.

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 wrapping film when mixing solutions during buffer exchange. In addition, unlike similar products from other vendors, reverse centrifugation is not required to recover the 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 molecular weights at least twice as high as 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.

Pierce Protein Concentrators, 0.5 mL

These concentrators are ideal for processing samples between 100 µL 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 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 MWCO and sample concentration or viscosity. The dead-stop volume is approximately 15 µL. Typical protein recovery is >90%.

Pierce Protein Concentrators, 6 mL

Ideal for processing samples between 2 mL and 6 mL, these 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 MWCO and sample concentration or viscosity. The dead-stop volume is approximately 30 µL. Typical protein recovery is >90%.

Pierce Protein Concentrators, 20 mL

These concentrators are ideal for processing samples between 5 mL and 20 mL. The 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 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 MWCO and sample concentration or viscosity. The dead-stop volume is approximately 50 µL. Typical protein recovery is >90%.

Pierce Protein Concentrators, 100 mL

Ideal for processing samples between 20 mL and 100 mL, these 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 use laboratory wrap to secure 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 MWCO and sample concentration or viscosity. The dead-stop volume is approximately 350 µL. Typical protein recovery is >90%.

Choose the right protein concentrator for your experiments 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 bacteria die, their cell membranes rupture and endotoxins (which are essential lipopolysaccharide (LPS) components of the cell walls) are released into the surrounding environment. Endotoxins are frequent contaminants of protein solutions derived from bioproduction, and are toxic to cells grown in tissue culture. Traditional endotoxin removal methods and their limitations are described in Table 4.

Products designed for efficient endotoxin removal

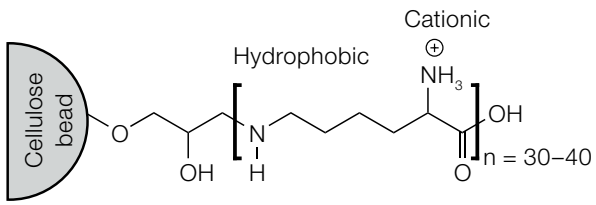
Ultrafiltration, polymyxin B affinity resin, and resin- or membrane-based chromatography are the traditional methods of endotoxin removal. All have limitations in protein recovery or endotoxin-binding capacity, or have toxicity concerns (Table 4). We offer a modified ε-poly-L-lysine [poly(ε-lysine)] affinity resin (Figure 8)—the ligand is a safe, nontoxic polymer of the natural amino acid lysine that is commonly used as a food preservative. This resin is available as a slurry to pack a custom column, or in convenient prepacked, single-use spin columns optimized for different sample volumes.

View resin performance data at
thermofisher.com/endotoxinspincolumns

Table 4. 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 proteins	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 protein will not be effectively removed. Technique also exerts strong physical forces on the protein	Successfully process proteins ranging from 12 to 150 kDa
Membrane-based chromatography	Reduced endotoxin-binding capacity compared to resin-based methods; nonreusable	Resin binds up to 2 x 10^6 EU* per mL of resin 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 (commonly used as a food preservative)

* One endotoxin unit (EU) is approximately 0.1 ng of endotoxin.



Poly(ε-lysine) resin

Figure 8. 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 matrix is masked by thorough derivatization of its interior and exterior surfaces with the poly(ε-lysine) ligand.

Available formats

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 prepacked, single-use spin columns optimized for different sample volumes (Table 5).

Highlights:

- **High capacity**—bind up to 2 x 10^6 EU per mL of resin, 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

Spin columns

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

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 of 10–15 mL/hr.

Learn more at thermofisher.com/endotoxinremoval

Table 5. Thermo Scientific™ endotoxin removal product formats.

	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

Protein research handbooks

Download free copies of our protein research handbooks at **thermofisher.com/proteinbooks**

Additional information and ordering

Visit our product pages for the latest application data, selection guides, and ordering information:

- Dialysis products: **thermofisher.com/dialysis**
- Zeba desalting products: **thermofisher.com/desalting**
- Detergent removal from peptides: **thermofisher.com/detergentremoval**
- Endotoxin removal: **thermofisher.com/endotoxinremoval**
- Protein concentrators: **thermofisher.com/concentrators**

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