Thermo Scientific Pierce High-Performance Dialysis, Desalting and Detergent Removal Technical Handbook

Featuring Thermo Scientific Slide-A-Lyzer Dialysis Cassettes

Version 2

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

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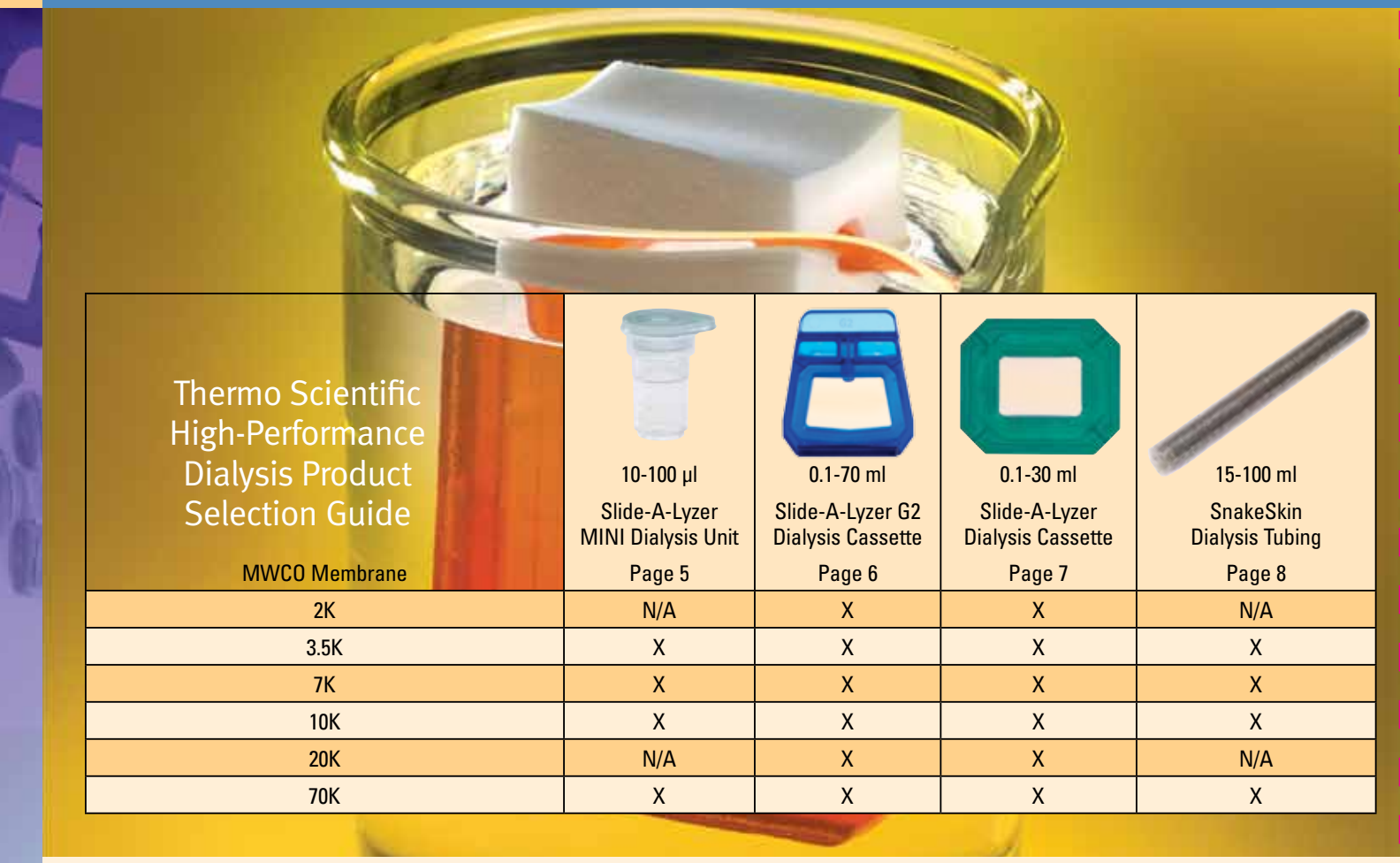

Thermo Scientific High-Performance Dialysis Product Selection Guide MWCO Membrane	 10-100 µl Slide-A-Lyzer MINI Dialysis Unit Page 5	 0.1-70 ml Slide-A-Lyzer G2 Dialysis Cassette Page 6	 0.1-30 ml Slide-A-Lyzer Dialysis Cassette Page 7	 15-100 ml SnakeSkin Dialysis Tubing Page 8
2K	N/A	X	X	N/A
3.5K	X	X	X	X
7K	X	X	X	X
10K	X	X	X	X
20K	N/A	X	X	N/A
70K	X	X	X	X

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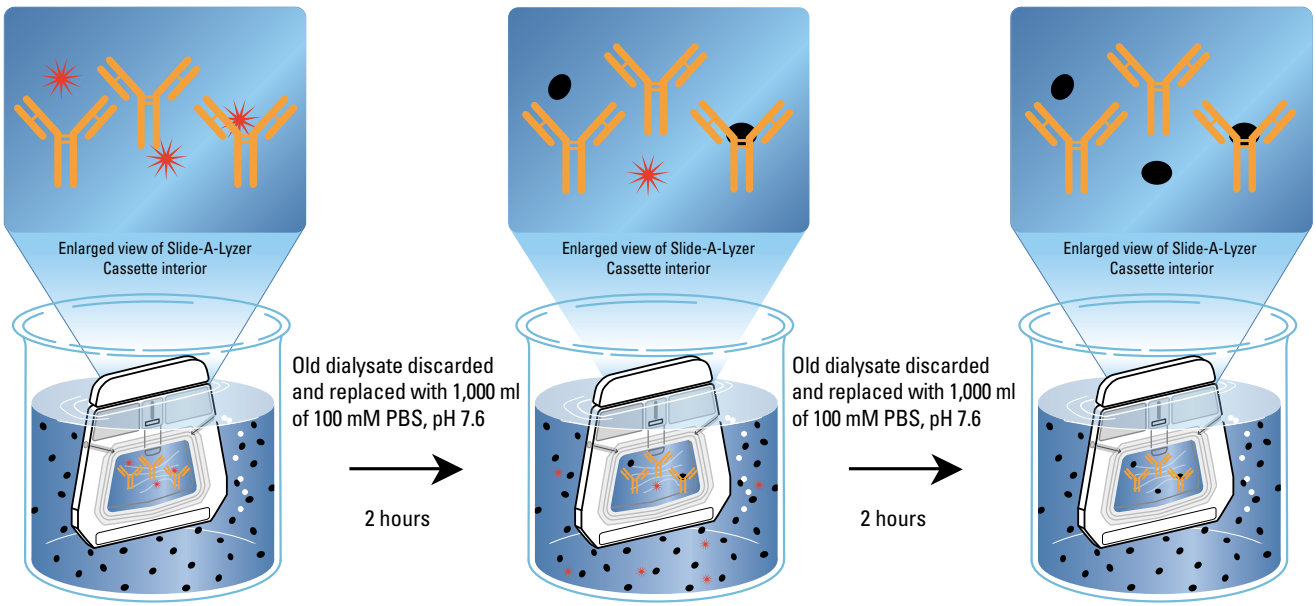
High-Performance Dialysis

Dialysis is a separation technique that gained popularity in life science laboratories during the 1950s. Research papers of that era described dialysis as a new, cutting-edge tool that scientists could use to unravel complex mixtures of biomacromolecules. Many of the dialysis theories established at that time are the cornerstones for contemporary products featured in this brochure. There are, however, two major differences between the dialysis tools of yesterday and today – preparation time and the amount of sample loss due to leaks. Early laboratory dialysis methods involved dedicating a significant amount of time to membrane preparation; Thermo Scientific Pierce Dialysis Products are essentially ready to use and resist sample leakage.

New developments in dialysis techniques were stagnant during the end of the 20th century, while ultrafiltration systems flourished fueled by advances in non-cellulose membranes and accessibility of bench-top centrifuges. Ultrafiltration via centrifugation was the established convention until we introduced the Slide-A-Lyzer Dialysis Cassette in 1994.



3 ml of 1 mg/ml IgG in 0.1 M Tris buffer, pH 7.4 inside a Slide-A-Lyzer Dialysis Cassette (10K MWCO) placed in 1,000 ml of 100 mM PBS, pH 7.6.

IgG would diffuse out if it could, but it is too large to enter the pores in the membrane; therefore, the amount of IgG inside the Cassette remains constant. The Tris buffer concentration drops below 0.01 M inside the Cassette as the Tris buffer diffuses out and the PBS buffer diffuses in.

IgG inside the Cassette remains constant. The Tris buffer inside the Cassette drops to near undetectable levels. The buffer inside the Cassette is 100 mM PBS, pH 7.6.

Dialysis: An Overview

Thermo Scientific Slide-A-Lyzer G2 Cassettes available in 0.1-0.5 ml, 0.5-3 ml, 15 ml, 30 ml and 70 ml.

10-100 µl Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit

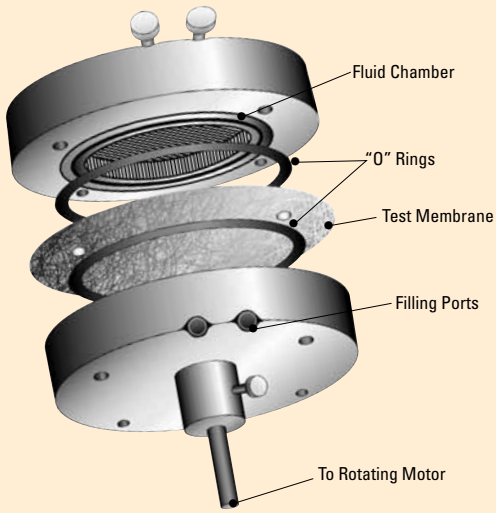
15-100 ml Thermo Scientific SnakeSkin Dialysis Tubing

Dialysis is the separation of small and large molecules in a solution by selective diffusion through a semipermeable membrane. Typically a sample containing a protein or nucleic acid will contain unwanted small molecular weight (MW) compounds such as a buffer salt (Tris, PBS, etc.), a reducing agent [dithiothreitol (DTT), β-mercaptoethanol (BME), etc.] or a preservative (sodium azide, thimerosol, etc.).

The sample is contained inside the dialysis membrane. A dialysate 200 to 300 times the volume of the sample is outside the dialysis membrane, which creates and maintains a concentration differential across the membrane. Once the liquid-to-liquid interface (sample on one side of the membrane and dialysate on the other) is initiated, all molecules will try to diffuse in either direction across the membrane to reach equilibrium. Dialysis (diffusion) will stop when equilibrium is achieved. Generally the rate of dialysis slows as equilibrium approaches, requiring the dialysate be changed after several hours to re-create the concentration differential that drives the dialysis process.

The membrane is the key to dialysis. The semipermeable membrane contains pores of a known size range that are large enough to let small MW compounds pass through, but restrict large MW compounds (e.g., proteins and nucleic acids). The ideal membrane is thin, has numerous pores of uniform diameter, and does not bind proteins and nucleic acids. What scientists have been using for decades is an extruded regenerated cellulose membrane that is close to an ideal membrane.

However, most scientists often assume too much chromatographic resolution associated with the membrane's molecular weight cutoff (MWCO).



We determine the MWCO of our dialysis membrane by using the rotating batch dialysis cell (see diagram[1] above). In the rotating cell, the membrane to be tested is held in place between two circular cavities of equal size. One side of the cell is partially filled with a solution containing a molecule of known MW. The other side is filled with an equal volume of buffer or saline. The solutions are mixed and kept in contact with the membrane by rotating the cell at a constant speed. The MW standard concentration in each half of the cell is measured after a fixed period of time and the percent retention is calculated. This type of system provides a more accurate MWCO determination than using ultrafiltration methods that measure hydraulic permeability or volumetric flux vs. pressure using saline or buffer alone.

Other important variables are sample and dialysate volume. The ideal scenario is to have a small sample volume and a large dialysate volume to maximize the concentration differential. The sample volume is important because subsequent applications have certain minimum volume requirements. However, after the minimum volume requirements are met, it is not advantageous to dialyze more sample than is needed. Depending on the surface area of a given sample, a small volume sample will dialyze much faster than a large volume sample. Not only is expending additional time wasteful, it can result in sample loss because the longer a sample is in contact with solid-phase surfaces, the more likely proteins or nucleic acids will nonspecifically bind or denature.

Reference

1. Klein, E., *et al.* DHEW Publication No. **77-1294**, p.17.

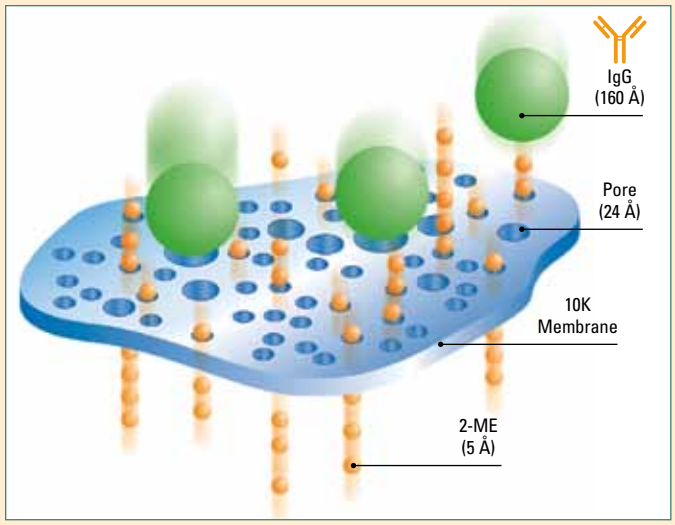


Figure 1. Diffusion of particles through a semi-permeable membrane.

Frequently Asked Questions About Dialysis

1) How precise is the MWCO?

The MWCO is reproducible, but not very precise. When choosing which MWCO membrane to use, it is advisable to have *both* the high MW compounds that you want to retain, and the low MW compounds that you want to diffuse out as far removed from the membrane's MWCO as possible.

Our dialysis products are available with 2K, 3.5K, 7K, 10K and 20K MWCO membranes. The retention profile exhibited is clearly distinct and reproducible for each MWCO membrane when testing compounds of known MW. We do not sell products with regenerated cellulose membranes with MWCOs below 2K and above 16K because they cannot be manufactured to our high-quality standards at this time.

2) Is stirring necessary?

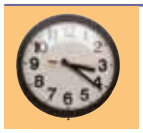
Stirring significantly decreases the dialysis time.

All membranes possess an inner skin, which experts have described as "seaweed-like," and an outer skin. There are no channels of a fixed diameter extending from the sample side through to the dialysate side. Instead, low MW compounds from the sample diffuse into the inner skin pores then through the membrane interior. These low MW compounds exit through a pore in the outer skin of the membrane, to a micro-environment called the Nernst layer. In this layer, which is approximately 200-300 molecules thick, low MW compounds are at a higher concentration in relation to the rest of the dialysate. Stirring, which efficiently breaks up the macro-environment outside the Nernst layer, quickly restores the concentration differential needed to drive the diffusion process.

3) Is temperature important?

Temperature is somewhat important because molecules move and diffuse faster at higher temperatures; however, maintaining the viability of your sample is the priority. So the typical range for dialysis is from ambient to cold-room temperatures.

4) When is my dialysis finished?



There is no easily measured dialysis endpoint. The goal is to reduce the concentration of low MW compounds to a level that will not interfere with subsequent steps in your experiment.

Standard practice has been as follows:

1) Dialyze for 2 hours at room temperature (RT),

2) Change the dialysate before dialyzing for another 2 hours at RT, and

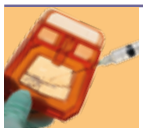
3) Change the dialysate again and dialyze for 1 hour to overnight in the cold room.

Thermo Scientific Pierce High-Performance Dialysis Products make the dialysis process faster than ever. The basic principle of the Slide-A-Lyzer MINI Dialysis Unit is to deposit a 10 µl sample (essentially a monolayer) on a dialysis membrane in contact with a dialysate that is 100,000 times larger than the sample volume. Small MW compounds have an extremely short (< 1 mm) migratory distance to exit the membrane. Also, with a gigantic concentration differential, the dialysis rate is fast (see page 4 Figures 1 and 2.)

5) Is membrane pretreatment necessary?

A short hydration is necessary for some MWCO membranes in the Slide-A-Lyzer Dialysis Cassette product line. Otherwise, the regenerated cellulose membranes are clean and require no pretreatment. A very small amount of either glycerine or sulfur may be present. These low MW compounds will diffuse out of the membrane and into the dialysate during the normal dialysis process. If necessary, these compounds may be dialyzed ahead of time but this is usually unnecessary.

6) When sample is injected into the Slide-A-Lyzer Dialysis Cassette, the membrane sometimes folds. What causes this?



Because the dialysis membrane is manufactured as a tube, the regenerated cellulose polymer has "memory" and wants to return to that shape even though the tube was cut into a flat membrane. Therefore, when a membrane is hydrated and the Cassette is filled, the membrane will stretch or pull differently with respect to the X-axis or Y-axis. Although this does have minor implications relative to surface area, these Slide-A-Lyzer Dialysis Cassettes will function just fine.

Thermo Scientific Slide-A-Lyzer MINI Dialysis Units

For sample volumes as small as 10 µl

Highlights:

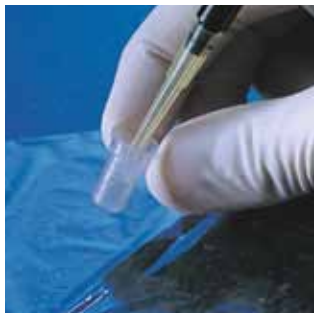
- **100% leak-tested**
  Patented design does not permit "wicking" that can occur in homemade devices
- **Very affordable**
- **Excellent sample recoveries**
  The Slide-A-Lyzer MINI Dialysis Unit generally recovers 9-10 µl after dialysis of a 10 µl sample
- **Time of dialysis drastically reduced**
  Converts 100 µl of pH 2.8 buffer to pH 9.4 dialyzing against 1 L bicarbonate buffer, pH 9.4 in less than 10 minutes

The Slide-A-Lyzer MINI Dialysis Unit is a small disposable cup made of polypropylene and regenerated cellulose. Sample is added and removed easily using a standard laboratory pipette. A float (sold separately) holds the Slide-A-Lyzer MINI Dialysis Unit upright, floating on the dialysate surface with the membrane in contact with the dialysate. Although the device's patented design is very simple, the easy-to-use Slide-A-Lyzer MINI Dialysis Unit is an invaluable tool for applications, like equilibrium competitive dialysis, for which only 10-100 µl samples are available.

1. Apply sample with a pipette.

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

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



4. Recover sample.

See ordering information on pages 9-12.

Dialysis Rate and Sample Recovery

The 3.5K Slide-A-Lyzer MINI Dialysis Unit was used for salt reduction analysis. Samples of 5-100 µl of 1 M NaCl were placed in the Slide-A-Lyzer MINI Dialysis Unit and dialyzed against 1 L of water for 10 minutes. To recover the smallest (5 µl and 10 µl) volumes from the Slide-A-Lyzer MINI Dialysis Unit, the device was tilted and gently tapped on the bottom edge to pool the sample. NaCl standards and samples were diluted in 50 ml ultrapure water and conductivity was measured (Cole-Parmer). The Slide-A-Lyzer MINI Dialysis Unit dialyzes efficiently (Figure 1). Dialysis rate of 100 µl of 5 M NaCl was also analyzed by conductivity (Figure 2). In a third experiment, the rate of pH exchange in the Slide-A-Lyzer MINI Dialysis Unit was determined and is also rapid. In less than 10 minutes, 100 µl of IgG Elution Buffer, pH 2.8 is converted to pH 9.4 by dialysis against 1 L of BupH™ Carbonate-Bicarbonate Buffer, pH 9.4 (data not shown).

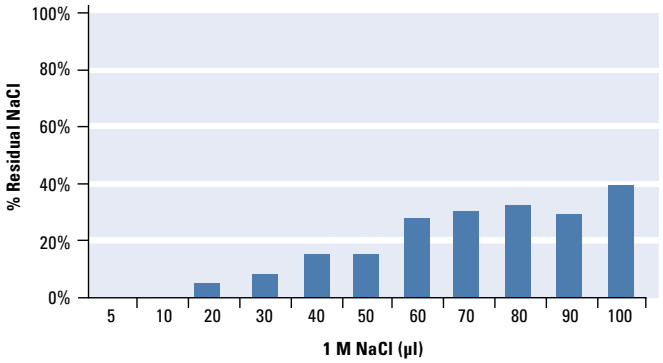


Figure 1. Dialysis efficiency in a Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit. After dialysis against water for 10 minutes, the residual NaCl is 0% for 5-10 µl samples, < 20% for 20-50 µl samples and < 40% for 60-100 µl samples, as measured with a conductivity meter.

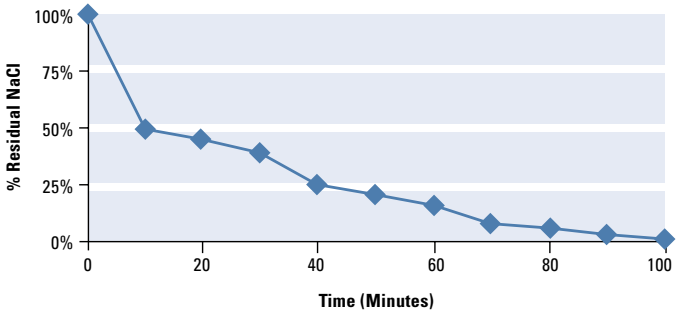


Figure 2. Dialysis time course in a Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit. 100 µl of 5 M NaCl was dialyzed for up to 100 minutes in a Slide-A-Lyzer MINI Dialysis Unit against 1 L of water. Within 10 minutes, only 50% of the NaCl remained; by 100 minutes, no NaCl remained.

Ren, J. and Chaires, J.B. (2001). Rapid screening of structurally selective ligand binding to nucleic acids. *Method Enzymol.* **340**, 99-108.

Thermo Scientific Slide-A-Lyzer G2 Dialysis Cassettes

New design for enhanced performance and ease-of-use

Slide-A-Lyzer G2 Dialysis Cassettes are available in a new 70 ml size.

Highlights:

- Pipette-accessible for easy sample loading and retrieval
- Self-floating chambers for buoyancy and vertical orientation during dialysis
- Designed to maintain the highest possible sample integrity and protection
- Fast and consistent dialysis with maximum sample recovery
- Rigorous quality testing for maximum consistency
- Ideal for removing low-molecular weight contaminants, performing buffer exchange and desalting

Join the thousands of researchers worldwide who save time and preserve their valuable samples by using Thermo Scientific Slide-A-Lyzer Dialysis Cassettes. The new generation of Slide-A-Lyzer Cassettes are flexible and easy-to-use. They are pipette-accessible, making it easy to add and remove your samples!

Dialysis is the most commonly used method for removing low-molecular weight solutes from macromolecules in solution or for buffer exchange. Dialysis separates sample components based on selective diffusion across a porous membrane. The membrane's pore size determines the molecular-weight cutoff (MWCO), which is characterized by the molecular weight at which 90% of the solute is retained. The permeability of a solute is dependent upon the shape of the molecule, the degree of hydration and its charge. Each of these characteristics may be influenced by the nature of the solvent, the pH and the ionic strength.

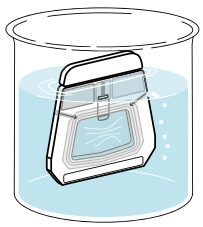
Traditional Tubing	vs.	Cassette
Slippery When Wet Flat tubing is difficult to handle and fill when wet.	sample handling	**Easy Handling and Secure Sample Delivery** No mess, no fuss.
Sample Loss Sample can easily be lost when tubing leaks or clamps slip off.	sample recovery	**Sample Protection** Highest possible sample protection with > 95% sample recovery.
Frequent Leaking Leaking into dialysate can compromise sample.	sample integrity	**No Leaking!** Sample remains intact with no contamination from surrounding dialysate.
Time-Consuming Typically dialyze overnight. Difficult to recover sample from wet tubing.	speed	**Fast and Efficient** High surface area/sample volume ratio will dialyze twice as fast as conventional tubing.

The new Slide-A-Lyzer G2 Cassette offers maximum efficiency, convenience and sample protection in one package. Sample loading and removal are easily accomplished by using a serological pipette or hypodermic needle (optional) attached to a syringe. The built-in air chamber provides sample buoyancy and vertical orientation of the cassette during dialysis. The cassette membrane is composed of low-binding regenerated cellulose for maximum sample recovery while maintaining maximum sample purity. The cassettes are available in five precise membrane MWCOs (2K, 3.5K, 7K, 10K and 20K) for dialyzing sample volumes from 100 µl up to 70 ml. The cassettes are manufactured using clean room conditions to ensure cassettes are contaminant-free.

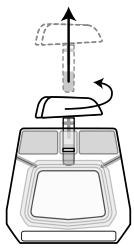
The new Slide-A-Lyzer G2 Dialysis Cassettes are now pipette-accessible. The cassettes are easy to load and offer a built-in air chamber for buoyancy and vertical orientation during dialysis.

See a demo of the new Slide-A-Lyzer G2 Cassettes at www.thermo.com/salG2

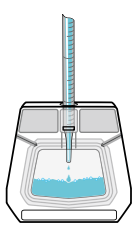
See ordering information on pages 9-13.



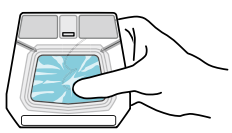
1. Immerse cassette in dialysis buffer for 2 minutes to hydrate membrane.



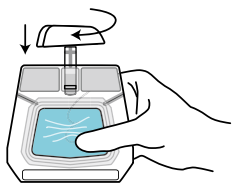
2. Remove cap by turning it counterclockwise and pullling upward.



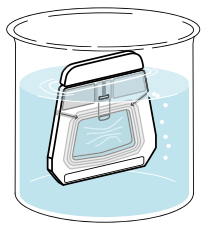
3. Add sample using a serological pipette.



4. Remove excess air by gently pressing the membrane.



5. While pressing membrane, replace cap and turn it clockwise to lock.



6. Immerse cassette in dialysis buffer and dialyze.

Thermo Scientific Slide-A-Lyzer Dialysis Cassettes

The original

Highlights:

- **> 95% sample recovery**
  Sample volume remains visible throughout dialysis
- **No knots or clamps to loosen and leak**
  Secure design prevents sample loss due to leaks
- **Rigid frame permits smooth sample withdrawal**
  Removing every last drop is easy – even for scientists who have never before performed dialysis
- **High surface area/sample volume ratio will dialyze twice as fast as dialysis via conventional tubing**
  Patented Cassette design spreads the sample over a large surface area and the double membrane promotes fast dialysis

1. Remove a Cassette from the protective pouch. Fill the Cassette cavity with your sample through one of the guide inlets in the corner of the Cassette. With the syringe still inserted into the cavity, draw up on the syringe to remove air.

2. Attach a flotation buoy and dialyze. Each buoy serves as an effective flotation device and also as a convenient bench-top stand for the Cassette.

3. Inject the Cassette chamber with air and withdraw your dialyzed sample from the Cassette.

The Slide-A-Lyzer Dialysis Cassette effectively and quickly dialyzes sample volumes from 100 µl to 30 ml. The Cassette's patented design, which provides a maximum surface area/sample volume ratio, allows for excellent sample recoveries. Unlike standard flat tubing, the innovative Cassette does not require the use of knots or clips that can lead to leaking and sample loss.

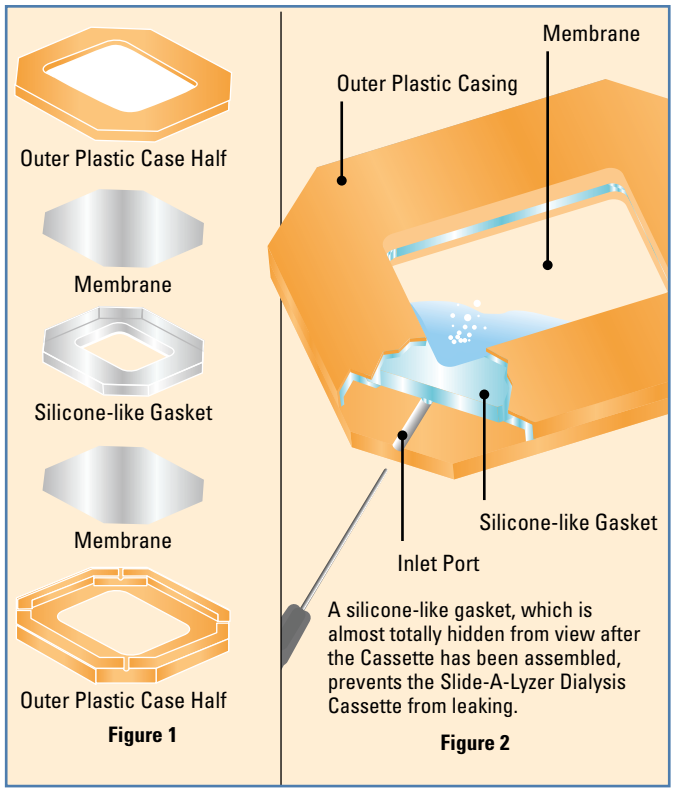


Figure 1 **Figure 2**

The Slide-A-Lyzer Dialysis Cassette (exploded view) looks like a sandwich (Figure 1). When all of the pieces are compressed together (Figure 2), the outer plastic case halves are welded together sonically, hermetically sealing an inner chamber that can be accessed only via a syringe needle inserted through the inlet port. Because the inert gasket is 10 mm wide, the needle path is sealed completely and tightly when the syringe is withdrawn.

Quantitative Sample Recovery

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 and conventional dialysis tubing to determine the volumes of recovery. Water volume recovered was determined gravimetrically. The following table summarizes the results:

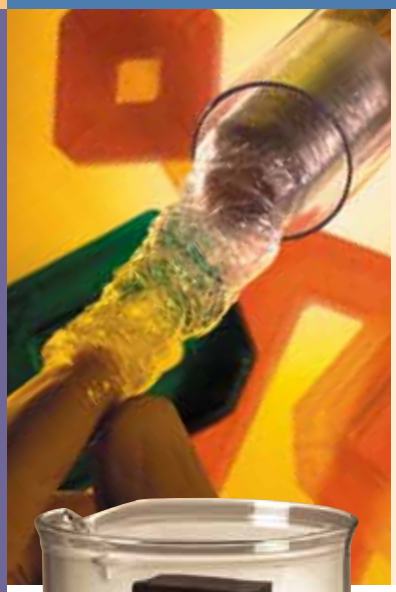
Average Sample Volume Recovery

Sample Volume Loaded	Thermo Scientific 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

See ordering information on pages 9-13.

Thermo Scientific SnakeSkin Dialysis Tubing

Avoid the hassles of large-sample dialysis using flat tubing



Tubing Specifications

Membrane Type: Regenerated cellulose

Glycerol Content: Varies with MWCO membrane

Sulfur Content: 0.1%-0.15%

Heavy Metals Content: Trace

Tubing Nominal Dry Thickness*

3.5K MWCO	1.0 mils
7K MWCO	1.2 mils
10K MWCO	0.9 mils

**1 mil = 25 microns*

Traditional flat dialysis tubing is difficult to open and often requires a presoak in water or buffer before it can be used. Handling the tubing after the presoak step can be messy and awkward. Thermo Scientific SnakeSkin Dialysis Tubing was developed to simplify large-sample dialysis. SnakeSkin Dialysis Tubing is open, regenerated cellulose dialysis tubing that is pleated (compressed) into a hollow stick. It is supplied in eight-inch sticks containing 35 feet of 22 mm internal diameter (I.D.) tubing, equivalent to 10.5 meters of 34 mm dry flat width tubing. SnakeSkin Dialysis Tubing can be used for 15-100 ml samples. The hydrated tubing will hold ~3.7 ml of sample per centimeter of length.

The pleated format of SnakeSkin Dialysis Tubing makes it easy to open and ready to use, streamlining dialysis preparation. To use it, a researcher simply pulls out the required length of tubing, cuts it off and applies a closure. The sample is then added through the other end of the dry tubing and the second closure is applied.

We recommend closure using SnakeSkin Dialysis Tubing Clips (sold separately). To use the clips, cut the desired length of tubing, fold one end over twice and apply a clip. Add the sample through the second end of the tubing, fold over twice and attach the second clip.

As an alternative to these clips, SnakeSkin Dialysis Tubing can also be closed with knots. Dip two to three inches of one end of the tubing into water or buffer and tie a knot in the wet membrane. (Dipping is required to assure a good seal at the knot point.) Add the sample to the open, dry end and tie a knot at this end. Because the sample quickly hydrates the membrane, there is no need to pre-wet the second end of the tubing.

The pleating process does not change the tubing's MWCO. Also, any low MW contaminants present are removed during the dialysis process. 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 MWCOs: 3.5K, 7K and 10K. The product is stored in its original packaging at room temperature, although refrigerated storage may also be used. Properly stored membrane is stable for at least one year.

Ordering Information

Product #	Description	MWCO	Pkg. Size
68035	SnakeSkin Dialysis Tubing	3.5K	22 mm dry I.D. x 35 feet*
68700	SnakeSkin Dialysis Tubing	7K	22 mm dry I.D. x 35 feet*
68100	SnakeSkin Dialysis Tubing	10K	22 mm dry I.D. x 35 feet*

**Equivalent to 10.5 meters of 34 mm dry flat width tubing.*

Product Accessories

Product #	Description	Pkg. Size.
68011	SnakeSkin Dialysis Tubing Clips	6/pkg.
66432	Slide-A-Lyzer Buoys for 12 ml Slide-A-Lyzer Cassettes	10/pkg.

Thermo Scientific Membrane Products – 2K MWCO

Specifications

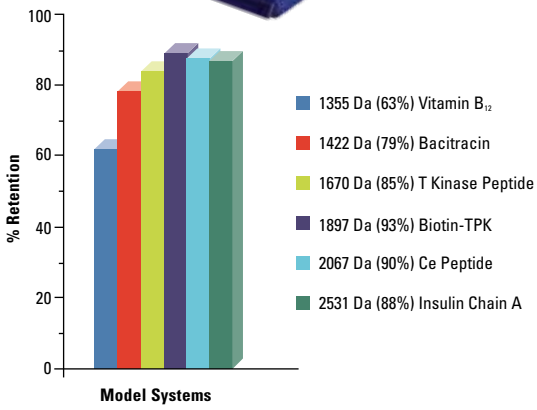
Membrane Composition: Regenerated cellulose synthesized by the Viscose method

Hydration Required Before Use: 2 minutes

Glycerol Content: None

Sulfur Content: 0.169%

Heavy Metals Content: Trace



Characterization of membrane pore size. Vitamin B_{12}, bacitracin, tyrosine kinase peptide 1, biotin-TPKs substrate, protein kinase Ce (PKCe) peptide substrate and insulin chain A model systems (0.5-1 mg/ml) in either saline or 0.2 M carbonate bicarbonate buffer pH 9.4 were dialyzed overnight (17 hours) at 4°C. The amount of retentate was estimated using either the Pierce BCA Protein Assay or absorption at 360 nm (for vitamin B_{12}).

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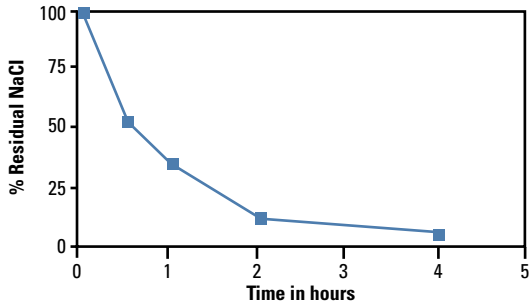


Desalting rate of the membrane for salts. Sodium chloride (1 M) in water was dialyzed at 4°C and the rate of removal of NaCl was determined by measuring the conductivity of the retentate at different time intervals.

Ordering Information

New Advanced Design Slide-A-Lyzer G2 Dialysis Cassettes

Product #	Description	Capacity	Pkg. Size
87717	Slide-A-Lyzer G2 Dialysis Cassette	0.1-0.5 ml	10/pkg.
87718	Slide-A-Lyzer G2 Dialysis Cassette	0.5-3 ml	10/pkg.
87719	Slide-A-Lyzer G2 Dialysis Cassette	15 ml	8/pkg.
87720	Slide-A-Lyzer G2 Dialysis Cassette	30 ml	6/pkg.
87721	Slide-A-Lyzer G2 Dialysis Cassette	70 ml	6/pkg.

NEW SIZE!

Slide-A-Lyzer MINI Dialysis Units

Product #	Description	Capacity	Pkg. Size
69580	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included*	10-100 µl	50/pkg.
69553	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included*	10-100 µl	250/pkg.

Slide-A-Lyzer Dialysis Cassettes

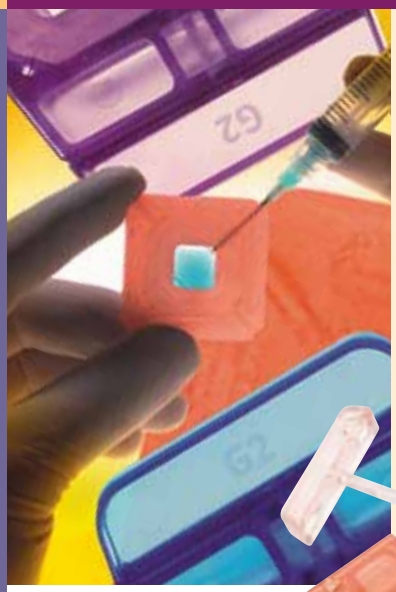
Product #	Description	Capacity	Pkg. Size
66205	Slide-A-Lyzer Dialysis Cassette	0.2-0.5 ml	10/pkg.
66203	Slide-A-Lyzer Dialysis Cassette	0.5-3 ml	10/pkg.
66212	Slide-A-Lyzer Dialysis Cassette	3-12 ml	8/pkg.
66230	Slide-A-Lyzer Dialysis Cassette	12-30 ml	6/pkg.

Product Accessories

Product #	Description	Pkg. Size
66430	Slide-A-Lyzer Buoys *Holds one 0.1-0.5 ml or 0.5-3 ml cassette.*	10/pkg.
66431	Slide-A-Lyzer Carousel Buoy *Holds ten 0.1-0.5 ml or 0.5-3 ml cassettes.*	1/pkg.
66432	Slide-A-Lyzer Buoys *Holds one 3-12 ml cassette.*	8/pkg.
66494	Slide-A-Lyzer Syringe (1 ml)*	10/pkg.
66490	Slide-A-Lyzer Syringe (5 ml)*	10/pkg.
66493	Slide-A-Lyzer Syringe (20 ml)* *Each syringe comes with 18-gauge 1-inch beveled needles.*	10/pkg.

** Can also be used with the new Slide-A-Lyzer G2 Dialysis Cassettes.*

Thermo Scientific Membrane Products – 3.5K MWCO



Specifications

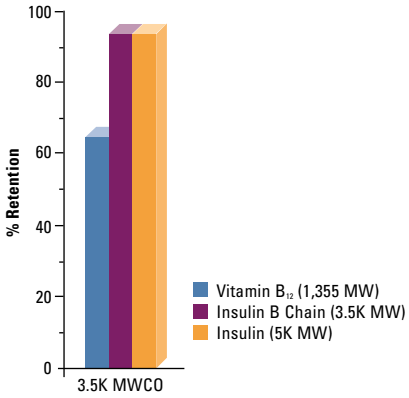
Membrane Composition: Regenerated cellulose synthesized by the Viscose method

Hydration Required Before Use: 30 seconds

Glycerol Content: Trace

Sulfur Content: 0.1%–0.15%

Heavy Metals Content: Trace



Sample retention by the 3.5K MWCO Thermo Scientific Slide-A-Lyzer Dialysis Cassette membrane. Known MW standards were dissolved at a concentration of 1 mg/ml in either 0.15 M sodium chloride or 0.2 M carbonate-bicarbonate buffer, pH 9.4 (Product # 28382). Rotating cells were assembled with the nominal 3.5K MWCO membranes. One half of the cell was filled with MW standard solution and the other half was filled with an equal volume of the plain diluent. Cells were rotated overnight at 100 rpm.

Ordering Information

New Advanced Design Slide-A-Lyzer G2 Dialysis Cassette

Product #	Description	Capacity	Pkg. Size
87722	Slide-A-Lyzer G2 Dialysis Cassette	0.1-0.5 ml	10/pkg.
87723	Slide-A-Lyzer G2 Dialysis Cassette	0.5-3 ml	10/pkg.
87724	Slide-A-Lyzer G2 Dialysis Cassette	15 ml	8/pkg.
87725	Slide-A-Lyzer G2 Dialysis Cassette	30 ml	6/pkg.
87726	Slide-A-Lyzer G2 Dialysis Cassette	70 ml	6/pkg.

NEW SIZE!

Slide-A-Lyzer MINI Dialysis Units

Product #	Description	Capacity	Pkg. Size
69558	Slide-A-Lyzer MINI Dialysis Units and Float *Sufficient caps are included.*	10-100 µl	10/pkg.
69550	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included.*	10-100 µl	50/pkg.
69552	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included.*	10-100 µl	250/pkg.

Slide-A-Lyzer Dialysis Cassettes

Product #	Description	Capacity	Pkg. Size
66333	Slide-A-Lyzer Dialysis Cassette	0.1-0.5 ml	10/pkg.
66335	Slide-A-Lyzer Dialysis Cassette Kit *Contains 10 cassettes, 10 buoys and 10 syringes.*	0.1-0.5 ml	Kit
66330	Slide-A-Lyzer Dialysis Cassette	0.5-3 ml	10/pkg.
66332	Slide-A-Lyzer Dialysis Cassette Kit *Contains 10 cassettes, 10 buoys and 10 syringes.*	0.5-3 ml	Kit
66110	Slide-A-Lyzer Dialysis Cassette	3-12 ml	8/pkg.
66107	Slide-A-Lyzer Dialysis Cassette Kit	3-12 ml	Kit
66130	Slide-A-Lyzer Dialysis Cassette	12-30 ml	6/pkg.

SnakeSkin Dialysis Tubing

Product #	Description	Pkg. Size
68035	SnakeSkin Dialysis Tubing *Equivalent to 10.5 meters of 34 mm dry width.*	22 mm dry I.D. x 35 ft

Product Accessories

Product #	Description	Pkg. Size
69588	Slide-A-Lyzer MINI Dialysis Unit Float *Holds 25 MINI Dialysis Units*	4/pkg.
66430	Slide-A-Lyzer Buoys *Each buoy holds one 0.1-0.5 ml or 0.5-3 ml cassette.*	10/pkg.
66431	Slide-A-Lyzer Carousel Buoy *Each buoy holds ten 0.1-0.5 ml or 0.5-3 ml cassettes.*	1/pkg.
66432	Slide-A-Lyzer Buoys *Each buoy holds one 3-12 ml cassette.*	8/pkg.
66494	Slide-A-Lyzer Syringe (1 ml capacity)*	10/pkg.
66490	Slide-A-Lyzer Syringe (5 ml capacity)*	10/pkg.
66493	Slide-A-Lyzer Syringe (20 ml capacity)* *Each syringe comes with 18-gauge 1-inch beveled needles.*	10/pkg.
68011	SnakeSkin Dialysis Tubing Clips	6/pkg.

** Can also be used with the new Slide-A-Lyzer G2 Dialysis Cassettes.*

Thermo Scientific Membrane Products – 7K MWCO

Specifications

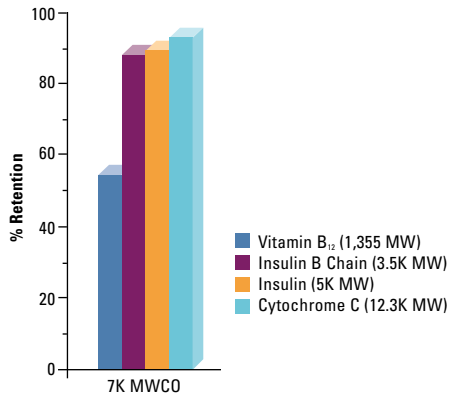
Membrane Composition: Regenerated cellulose synthesized by the Viscose method

Hydration Required Before Use: 30 seconds for low-volume samples

Glycerol Content: 13%

Sulfur Content: 0.1%–0.15%

Heavy Metals Content: Trace



Sample retention by the 7K MWCO Thermo Scientific Slide-A-Lyzer Dialysis Cassette membrane. Known MW standards were dissolved at a concentration of 1 mg/ml in either 0.15 M sodium chloride or 0.2 M carbonate-bicarbonate buffer, pH 9.4 (Product # 28382). Rotating cells were assembled with the nominal 7K MWCO membranes. One half of the cell was filled with MW standard solution and the other half was filled with an equal volume of the plain diluent. Cells were rotated overnight at 100 rpm.

Ordering Information

New Advanced Design Slide-A-Lyzer G2 Dialysis Cassettes

Product #	Description	Capacity	Pkg. Size
87727	Slide-A-Lyzer G2 Dialysis Cassette	0.1-0.5 ml	10/pkg.
87728	Slide-A-Lyzer G2 Dialysis Cassette	0.5-3 ml	10/pkg.

Slide-A-Lyzer MINI Dialysis Units

Product #	Description	Capacity	Pkg. Size
69560	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included.*	10-100 µl	50/pkg.
69562	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included.*	10-100 µl	250/pkg

Slide-A-Lyzer Dialysis Cassettes

Product #	Description	Capacity	Pkg. Size
66373	Slide-A-Lyzer Dialysis Cassette	0.1-0.5 ml	10/pkg.
66375	Slide-A-Lyzer Dialysis Cassette Kit *Contains 10 cassettes, 10 buoys and 10 syringes.*	0.1-0.5 ml	Kit
66370	Slide-A-Lyzer Dialysis Cassette	0.5-3 ml	10/pkg.
66372	Slide-A-Lyzer Dialysis Cassette Kit *Contains 10 cassettes, 10 buoys and 10 syringes.*	0.5-3 ml	Kit
66710	Slide-A-Lyzer Dialysis Cassette	3-12 ml	8/pkg.
66707	Slide-A-Lyzer Dialysis Cassette Kit *Contains 8 cassettes, 8 buoys and 10 syringes.*	3-12 ml	Kit

SnakeSkin Dialysis Tubing

Product #	Description	Pkg. Size
68700	SnakeSkin Dialysis Tubing *Equivalent to 10.5 meters of 34 mm dry width.*	22 mm dry I.D. x 35 ft

Product Accessories

Product #	Description	Pkg. Size
69588	Slide-A-Lyzer MINI Dialysis Unit Float *Holds 25 MINI Dialysis Units*	4/pkg.
66430	Slide-A-Lyzer Buoys *Each buoy holds one 0.1-0.5 ml or 0.5-3 ml cassette.*	10/pkg.
66431	Slide-A-Lyzer Carousel Buoy *Each buoy holds ten 0.1-0.5 ml or 0.5-3 ml cassettes.*	1/pkg.
66432	Slide-A-Lyzer Buoys *Each buoy holds one 3-12 ml cassette.*	8/pkg.
66494	Slide-A-Lyzer Syringe (1 ml capacity)*	10/pkg.
66490	Slide-A-Lyzer Syringe (5 ml capacity)*	10/pkg.
66493	Slide-A-Lyzer Syringe (20 ml capacity)* *Each syringe comes with 18-gauge 1-inch beveled needles.*	10/pkg.
68011	SnakeSkin Dialysis Tubing Clips	6/pkg.

** Can also be used with the new Slide-A-Lyzer G2 Dialysis Cassettes.*

Specifications

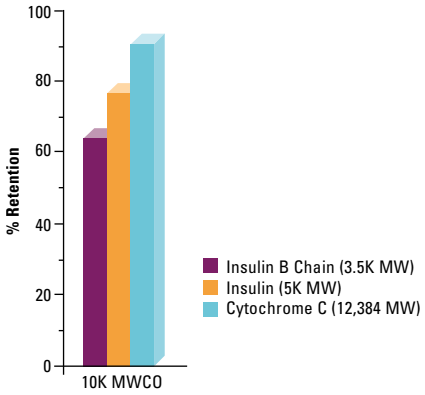
Membrane Composition: Regenerated cellulose synthesized by the Viscose method

Hydration Required Before Use: 30 seconds

Glycerol Content: 21%

Sulfur Content: 0.05%

Heavy Metals Content: Trace



Sample retention by the 10K MWCO Thermo Scientific Slide-A-Lyzer Dialysis Cassette membrane. Known MW standards were dissolved at a concentration of 1 mg/ml in either 0.15 M sodium chloride or 0.2 M carbonate-bicarbonate buffer, pH 9.4 (Product # 28382). Rotating cells were assembled with the nominal 10K MWCO membranes. One half of the cell was filled with MW standard solution and the other half was filled with an equal volume of the plain diluent. Cells were rotated overnight at 100 rpm.

Ordering Information

New Advanced Design Slide-A-Lyzer G2 Dialysis Cassette

Product #	Description	Capacity	Pkg. Size
87729	Slide-A-Lyzer G2 Dialysis Cassette	0.1-0.5 ml	10/pkg.
87730	Slide-A-Lyzer G2 Dialysis Cassette	0.5-3 ml	10/pkg.
87731	Slide-A-Lyzer G2 Dialysis Cassette	15 ml	8/pkg.
87732	Slide-A-Lyzer G2 Dialysis Cassette	30 ml	6/pkg.
87733	Slide-A-Lyzer G2 Dialysis Cassette	70 ml	6/pkg.

NEW SIZE!

Slide-A-Lyzer MINI Dialysis Units

Product #	Description	Capacity	Pkg. Size
69574	Slide-A-Lyzer MINI Dialysis Unit Plus Microtubes *Sufficient caps are included.*	10-100 µl	10/pkg.
69570	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included.*	10-100 µl	50/pkg.
69572	Slide-A-Lyzer MINI Dialysis Unit *Sufficient caps are included.*	10-100 µl	250/pkg.
69576	Slide-A-Lyzer MINI Dialysis Unit Plus Float *Sufficient caps are included.*	10-100 µl	Kit/10 units

Slide-A-Lyzer Dialysis Cassettes

Product #	Description	Capacity	Pkg. Size
66383	Slide-A-Lyzer Dialysis Cassette	0.1-0.5 ml	10/pkg.
66384	Slide-A-Lyzer Dialysis Cassette	0.1-0.5 ml	5 x 10/pkg.
66385	Slide-A-Lyzer Dialysis Cassette Kit *Contains 10 cassettes, 10 buoys and 10 syringes.*	0.1-0.5 ml	Kit
66380	Slide-A-Lyzer Dialysis Cassette	0.5-3 ml	10/pkg.
66381	Slide-A-Lyzer Dialysis Cassette	0.5-3 ml	5 x 10/pkg.
66382	Slide-A-Lyzer Dialysis Cassette Kit *Contains 10 cassettes, 10 buoys and 10 syringes.*	0.5-3 ml	Kit
66810	Slide-A-Lyzer Dialysis Cassette	3-12 ml	8/pkg.
66811	Slide-A-Lyzer Dialysis Cassette	3-12 ml	5 x 10/pkg.
66807	Slide-A-Lyzer Dialysis Cassette Kit *Contains 8 cassettes, 8 buoys and 10 syringes.*	3-12 ml	Kit
66830	Slide-A-Lyzer Dialysis Cassette	12-30 ml	6/pkg.

λ Irradiated 10K MWCO Membrane

Product #	Description	Capacity	Pkg. Size
66454	Slide-A-Lyzer Dialysis Cassette	0.1-0.5 ml	10/pkg.
66455	Slide-A-Lyzer Dialysis Cassette	0.5-3 ml	10/pkg.
66453	Slide-A-Lyzer Dialysis Cassette	3-12 ml	8/pkg.
66456	Slide-A-Lyzer Dialysis Cassette	12-30 ml	8/pkg.

SnakeSkin Dialysis Tubing

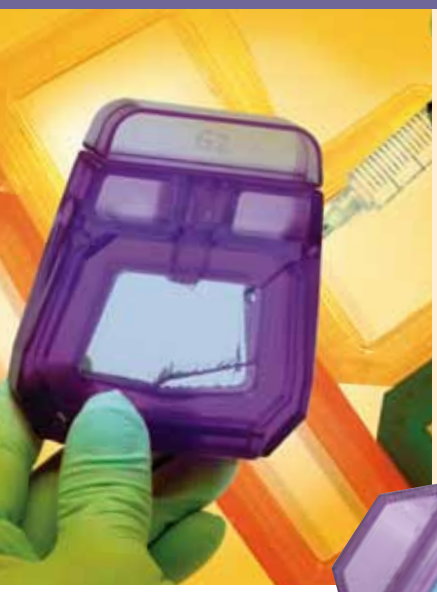
Product #	Description		Pkg. Size
68100	SnakeSkin Dialysis Tubing *Equivalent to 10.5 meters of 34 mm dry width.*	12-30 ml	22 mm dry I.D. x 35 ft

Product Accessories

Product #	Description	Pkg. Size
69588	Slide-A-Lyzer MINI Dialysis Unit Float	4/pkg.
66430	Slide-A-Lyzer Buoys *Holds one 0.1-0.5 ml or 0.5-3 ml cassette.*	10/pkg.
66431	Slide-A-Lyzer Carousel Buoy *Holds ten 0.1-0.5 ml or 0.5-3 ml cassettes.*	1/pkg.
66432	Slide-A-Lyzer Buoys *Holds one 3-12 ml cassette.*	8/pkg.
66494	Slide-A-Lyzer Syringe (1 ml capacity)*	10/pkg.
66490	Slide-A-Lyzer Syringe (5 ml capacity)*	10/pkg.
66493	Slide-A-Lyzer Syringe (20 ml capacity)* *Each syringe comes with 18-gauge 1-inch beveled needles.*	10/pkg.
68011	SnakeSkin Dialysis Tubing Clips	6/pkg.

** Can also be used with the new Slide-A-Lyzer G2 Cassettes.*

Thermo Scientific Membrane Products – 20K MWCO



Specifications

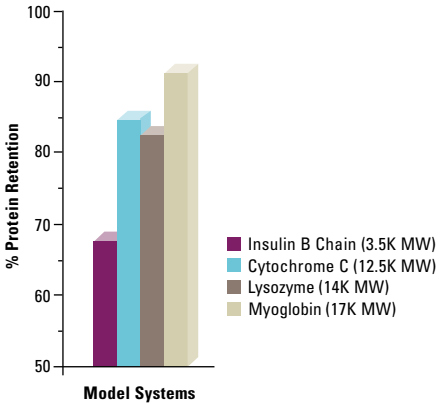
Membrane Composition: Regenerated cellulose synthesized by the Viscose method

Hydration Required Before Use: 2 minutes

Glycerol Content: None

Sulfur Content: 0.04%

Heavy Metals Content: Trace



Characterization of membrane pore size. Insulin B chain, cytochrome C, lysozyme and myoglobin were dialyzed overnight (17 hours) at 4°C in PBS pH 7.4. The amount of retentate was estimated using the Pierce BCA Protein Assay.

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Ordering Information

New Advanced Design
Slide-A-Lyzer G2 Dialysis Cassette

Product #	Description	Capacity	Pkg. Size
87734	Slide-A-Lyzer G2 Dialysis Cassette	0.1-0.5 ml	10/pkg.
87735	Slide-A-Lyzer G2 Dialysis Cassette	0.5-3 ml	10/pkg.
87736	Slide-A-Lyzer G2 Dialysis Cassette	15 ml	8/pkg.
87737	Slide-A-Lyzer G2 Dialysis Cassette	30 ml	6/pkg.
87738	Slide-A-Lyzer G2 Dialysis Cassette	70 ml	6/pkg.

NEW SIZE!

Slide-A-Lyzer Dialysis Cassettes

Product #	Description	Capacity	Pkg. Size
66005	Slide-A-Lyzer Dialysis Cassette	0.1-0.5 ml	10/pkg.
66003	Slide-A-Lyzer Dialysis Cassette	0.5-3 ml	10/pkg.
66012	Slide-A-Lyzer Dialysis Cassette	3-12 ml	8/pkg.
66030	Slide-A-Lyzer Dialysis Cassette	12-30 ml	6/pkg.

Product Accessories

Product #	Description	Pkg. Size
69588	Slide-A-Lyzer MINI Dialysis Unit Float	4/pkg.
66430	Slide-A-Lyzer Buoys *Holds one 0.1-0.5 ml or 0.5-3 ml cassette.*	10/pkg.
66431	Slide-A-Lyzer Carousel Buoy *Holds ten 0.1-0.5 ml or 0.5-3 ml cassettes.*	1/pkg.
66432	Slide-A-Lyzer Buoys *Holds one 3-12 ml cassette.*	8/pkg.
66494	Slide-A-Lyzer Syringe (1 ml capacity)*	10/pkg.
66490	Slide-A-Lyzer Syringe (5 ml capacity)*	10/pkg.
66493	Slide-A-Lyzer Syringe (20 ml capacity)* *Each syringe comes with 18-gauge 1-inch beveled needles.*	10/pkg.
68011	SnakeSkin Dialysis Tubing Clips	6/pkg.

** Can also be used with the new Slide-A-Lyzer G2 Dialysis Cassettes.*

The RED Device for Rapid Equilibrium Dialysis

A transforming technology for plasma protein-binding assays

Determining the extent to which a molecule binds to plasma proteins is a critical phase of drug development because it influences compound dosing, efficacy, clearance rate and potential for drug interactions. This determination is enabled by equilibrium dialysis, an accepted standard method for reliable estimation of the nonbound drug fraction in plasma. Although it is the preferred method, equilibrium dialysis is labor-intensive, time-consuming, cost-prohibitive and difficult to automate. The RED Device† for rapid equilibrium dialysis was developed in close association with the pharmaceutical industry to specifically address these issues, accelerating lead optimization and reducing attrition rate.

The RED System consists of disposable tube inserts and a 96-well Teflon® Base Plate. The unique design of the base plate provides compatibility with automated liquid handling systems while the large dialysis surface area of the tube inserts accelerates equilibrium.

The RED Device has been extensively validated for plasma-binding assays producing results consistent with those reported in the literature (Table 1). Using the RED Device to measure Warfarin binding to plasma (human and rat) proteins at two concentrations of 1 and 10 µM, the RED Device produced results with minimal intra-experimental variability (Figure 1). The RED System offers significant improvements in the ease of use, time requirements, versatility and product reliability compared to competitors (Table 2).

The RED Device Enables:

- Determination of free vs. bound drug to plasma proteins
- Pharmacokinetics studies
- Formulation of drug dosage for *in vivo* studies
- Drug-to-drug interaction studies
- Selection criteria during drug lead optimization

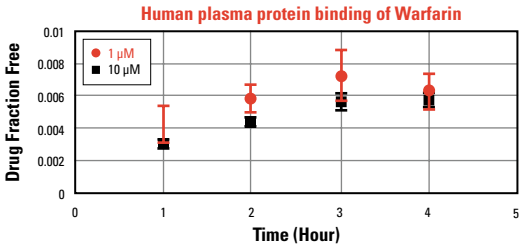
Highlights:

- **Ease of use**
  Disposable tubes require no presoaking, assembly or specialized equipment
- **Short incubation time**
  Equilibrium can be reached in as few as three hours as a result of the high membrane surface-to-volume ratio
- **96-well format**
  Suitable for automated liquid handlers
- **Flexible**
  Can be used for the desired number of assays (one to 48 assays/plate) without wasting the entire plate
- **Robust**
  Compartmentalized design eliminates potential for crosstalk or leakage
- **Reproducible and accurate**
  Validated for plasma-binding assays, producing results consistent with those reported in the literature (Table 1)
- **Versatile**
  The high-grade Teflon Base Plate is chemically inert, eliminating nonspecific binding and risk of contamination
- **Validated**
  Each lot is functionally tested in a protein-binding assay for guaranteed performance
- **Convenient**
  The RED Device membrane has a MWCO of 8K; other MWCO membranes are available upon request

Table 1. Comparison of results obtained using the RED Device with values reported in the literature.

	% Bound	
Compound	**Literature Value**	**RED Device**
Ranitidine[1]	10-19	17
Propranolol[2]	87-96	84
Warfarin[3]	99	99
Naproxen[1]	99	99

1. Jusko, W.J. and Gretch, M. (1976). Plasma and tissue protein binding of drugs in pharmacokinetics. *Drug Metab. Rev.,* **5(1),** 43-140.
2. Colangelo, P.M., *et al.* (1992). Age and propranolol stereoselective disposition in humans. *Clin. Pharmacol. Ther.,* **51**, 489-94.
3. Chan, E., *et al.* (1994). Disposition of warfarin enantiomers and metabolites in patients during multiple dosing with rac-warfarin. *Brit. J. Clin. Pharmacol.,* **36**, 563-569.



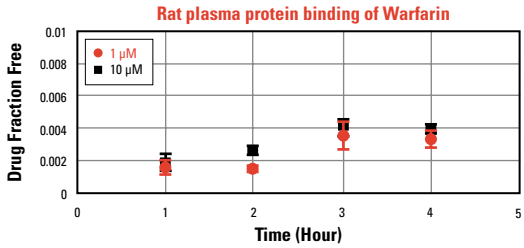


Figure 1. The RED Device binds plasma proteins. More than 99% of Warfarin was consistently bound to plasma protein showing minimal intra-experimental variability. Three replicate RED Device inserts were set up for each tested time point. Warfarin solutions at 1 µM or 10 µM were made in the plasma of choice and added to the insert sample chamber. PBS was added to the buffer chamber. At each time point (1, 2, 3 and 4 hours), 50 µl was removed from the plasma and the buffer chambers and transferred to separate wells of a deep well plate. After all the time points were collected, 50 µl of blank plasma was added to every buffer sample and 50 µl buffer was added to every plasma sample. After precipitation buffer was added, vortexed and centrifuged, the supernatants were analyzed by LC/MS/MS (API4000). A standard curve of the drug of interest was prepared along with the samples. The concentration of each sample was determined from the standard curve.

Table 2. A comparison of critical attributes for equilibrium devices.††

Device (Source)	Hours to reach Equilibrium	Leakage	Disposable	Labor Intensity	Automation Accessible	Volume Shift
RED (Rapid Equilibrium Dialysis) Device (Thermo Scientific)	4	None	Yes	•	Yes	None
Multi-Equilibrium Dialyzer (Harvard Apparatus)	3-4	Minimum	No	••••	No	Minimum
96-well Equilibrium DIALYZER (Harvard Apparatus)	16	20%	Yes	•••	Possible	Yes
96-well Micro Equilibrium Dialysis Block (HTDialysis, LLC)	6	Some	No	•••	Possible	Yes
24-Multiwell Dialysis (BD Biosciences)	24	Not measured	Yes	••	Possible	Not measured

†† Li, S.,[1] Xiong, B.[2] Huang, T.,[2] Li, L.,[2] Donovan, J.,[3] Lee, F.,[1] Yu, S.,[1] Miwa, G.[1] and Yang, H.[1] Validation of a novel rapid equilibrium dialysis (RED) device for high throughput plasma protein binding determination. 1. DMPK/Drug Safety & Disposition; 2. Linden Bioscience, 35A Cabot Road, Woburn, MA 01801, USA; and 3. Process Technology, Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA 02139 USA.

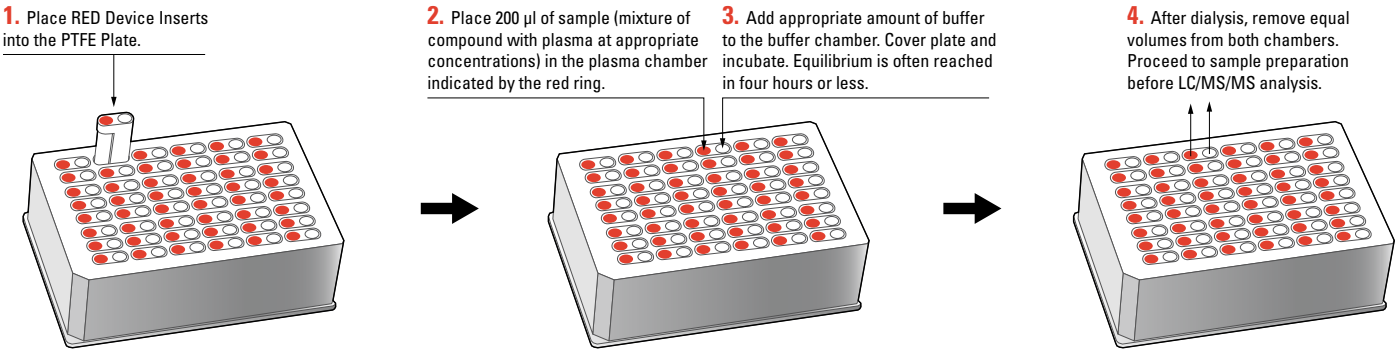


Figure 2. Schematic protocol for the RED Device.

Ordering Information

Product #	Description	Pkg. Size
89809	RED Device Inserts	50/pack
89810	RED Device Inserts	250/case
89811	PTFE Base Plate	1 plate
89812	RED Device Insert Removal Tool	1 remover

† The RED Device is manufactured by Linden Bioscience. Patent Pending on RED Device by Linden Bioscience.

Competition Rapid Equilibrium Dialysis

Quickly perform accurate and reproducible drug tissue-binding studies

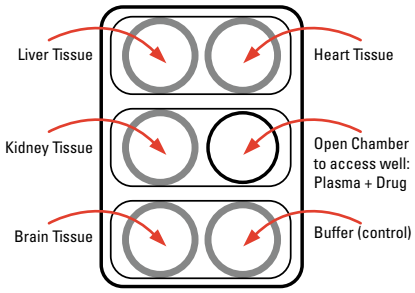
A critical phase of drug development is determining the extent to which a drug is distributed between plasma and specific tissues, which determines compound dosing, efficacy, clearance rate and the potential for drug interactions or tissue damage. The Thermo Scientific Competition Rapid Equilibrium Dialysis (Competition RED) Device[1] is an expansion of our popular rapid equilibrium dialysis (RED) product line and was developed in association with pharmaceutical laboratories to better model *in vivo* drug-tissue interactions.

Highlights:

- **Easy to use** – disposable inserts are supplied ready to use (i.e., no presoaking, assembly or specialized equipment necessary)
- **Short incubation time** – equilibrium can be reached as fast as two to six hours
- **Flexible format** – variable well sizes enable small-molecule partitioning studies of two to 16 tissue or protein samples
- **Versatile** – base plate is composed of chemically inert high-grade PTFE, eliminating nonspecific binding and contamination risks
- **Validated** – each lot is functionally tested in a protein-binding assay for guaranteed performance

Because of time, high cost and regulation of animal dosing and testing, *in vitro* models are highly desired for pre-screening compounds. A wide variety of cell, membrane and tissue section or distribution and equilibrium dialysis-based pre-screening methods have been developed and implemented to varying degrees of success. Currently, tissue-plasma drug-binding studies using equilibrium dialysis examine the binding affinity of a small molecule between plasma and one tissue homogenate at a time. In an individual tissue-plasma binding study, a drug may result in significant binding but the binding profile will be different in a complex system.

Within the body, a drug distributes and reaches equilibrium based on competitive interactions with all encountered fluids and tissues. The Competition RED Device is specifically designed to mimic, in the best possible way, these multiple interactions in an *in vitro* assay. Although, competition RED screening does not replace animal testing, performing a pre-screen accelerates and improves drug candidate selection and minimizes cost by potentially identifying drugs with overly strong or weak binding to specific tissues. The Competition RED System consists of disposable dialysis tube inserts and a reusable PTFE base plate, which minimizes waste while providing experimental design flexibility. The unique base plate design allows placement of two to 16 dialysis chambers into a common well enabling researchers to perform several experiments simultaneously. The Competition RED System has a standard 96-well plate footprint with 9 x 9 mm well spacing. Additionally, the small volume and large dialysis surface area of the tube inserts allows rapid dialysis, achieving equilibrium in two to four hours with high levels of reproducibility and accuracy. The device inserts have a molecular-weight cutoff (MWCO) of 12,000.



Example device setup for monitoring drug partitioning. Each 10-pack of Competition RED Inserts contains eight dual-chamber inserts and two single-chamber inserts. The open chamber in the single-chamber inserts enables direct access to the sample in the base plate well without disassembling the device.

Applications:

- Hit-to-lead selection of new chemical entities for preclinical studies
- Preliminary drug candidate screening in ADME-Tox studies – *in vitro* screening of drug partitioning between plasma and multiple tissues before *in vivo* studies
- Determining formulation of drug dosage for *in vivo* studies
- Competitive binding and dissociation constant determination for small molecules versus multiple targets

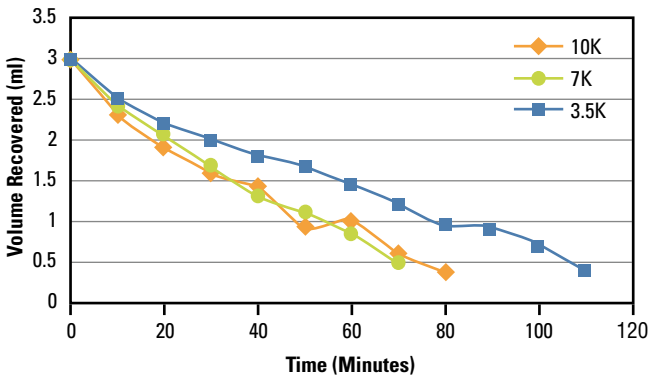
Ordering Information

Product #	Description	Pkg. Size
90087	Competition RED Inserts	10/pkg.
90088	Competition RED Inserts	50/pkg.
90085	Competition RED Base Plate	1 unit

Thermo Scientific Slide-A-Lyzer Concentrating Solution

Thermo Scientific Slide-A-Lyzer Concentrating Solution is a proprietary, hygroscopic, high MW compound that pulls water through dialysis membrane quickly. Other concentrating solutions concentrate and contaminate samples with a compound of similar MW that is difficult to remove by dialysis or other means. These contaminants absorb strongly at 280 nm, distorting protein measurements using the tyrosine absorption method. The Slide-A-Lyzer Concentrating Solution special formulation is free of low MW compounds that could cross the membrane to contaminate the sample.

Many samples will take on water or buffer during the dialysis process. To return the sample to its original concentration, or to concentrate it even further, the Slide-A-Lyzer Concentrating Solution is ideal. To concentrate the sample, the Slide-A-Lyzer Dialysis Cassette containing the sample is placed in a small plastic bag containing the concentrating solution. By diffusion, water and other small molecules are drawn out of the cassette, into the bag. The large molecular size of the concentrating solution prevents it from crossing the membrane and entering the cassette. Therefore, a one-way flow of water and other small molecules out of the Cassette results in concentration of the sample.



The Slide-A-Lyzer Concentrating Solution quickly reduces a starting volume of 3 ml of sample inside the Slide-A-Lyzer Dialysis Cassette to 0.5 ml in about 50 minutes. This is comparable to other concentration methods such as centrifuge-driven membrane devices.

Highlights:

- **Dialysis and concentration occur in one device**
  Avoids protein loss by using a single device
- **Faster concentration**
  A starting volume of 3 ml is reduced to 0.5 ml in about 75-80 minutes
- **Easy to use**
  Just pour the Slide-A-Lyzer Concentrating Solution into the small plastic bag provided and drop in the Slide-A-Lyzer Dialysis Cassette containing the sample
- **Improved formulation and protocols**
  Improved product makes concentration easier with rocking-platform protocols
- **The process can be monitored**
  Because both the concentrating solution and the bag are clear, the sample concentration can be easily monitored, something that is not possible with closed-system centrifuge-type devices

Ordering Information

Product #	Description	Pkg. Size
66528	Slide-A-Lyzer Concentrating Solution *For use with 0.5-3 ml cassettes.*	200 ml
66529	Slide-A-Lyzer Concentrating Solution *For use with 3-30 ml cassettes.*	500 ml
66530	Slide-A-Lyzer Concentrating Solution *For use with Slide-A-Lyzer MINI Dialysis Units*	25 ml

Concentrating with the Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit

Slide-A-Lyzer Concentrating Solution works on even very small samples using the Slide-A-Lyzer MINI Dialysis Unit. Samples from 10 to 100 µl are placed in the Slide-A-Lyzer MINI Dialysis Unit and then placed in a microcentrifuge tube that contains Slide-A-Lyzer Concentrating Solution at a minimum ratio of 3:1 (Concentrating Solution to sample).

Detergent Removal Resin and Spin Columns

Effectively remove detergents from protein and peptide samples

Detergents or surfactants are important for solubilizing, stabilizing and disaggregating proteins; however, detergents interfere with many downstream analysis methods. Therefore, it is often crucial to remove non-bound detergents before using proteins samples for ELISA, isoelectric focusing or mass spectrometry (MS). Unfortunately, typical sample clean-up methods, such as dialysis and size-exclusion chromatography, are often ineffective at removing detergents. We developed an efficient and rapid spin-column method (Figure 1) for removing detergents from protein and peptide solutions. The Thermo Scientific Pierce Detergent Removal Resin efficiently removes high concentrations of detergents from 0.01-1 ml samples with minimal sample loss.

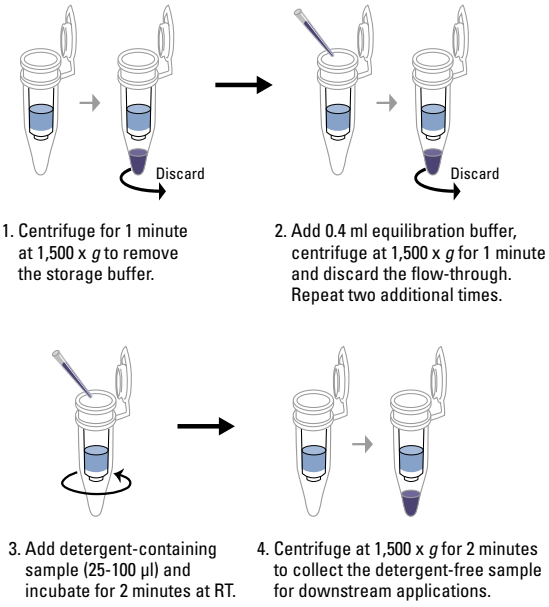


Figure 1. Protocol summary for Thermo Scientific Pierce Detergent Removal Spin Columns (0.5 ml).

Results and Discussion

We processed protein samples containing a wide range of detergents with the Pierce Detergent Removal Resin. Detergents at concentrations from 1 to 5% were effectively removed with generally > 90% protein recovery (Table 1).

Table 1. Detergents are effectively removed with high protein recovery.*

Detergent and Removable Concentration (%)	Detergent Removal (%)	BSA Recovery (%)
SDS (2.5)	99	95
Sodium deoxycholate (5)	99	100
CHAPS (3)	99	90
Octyl glucoside (5)	99	90
Octyl thioglucoside (5)	99	95
Lauryl maltoside (1)	98	99
Triton® X-100 (2)	99	87
Triton X-114 (2)	95	100
NP-40 (1)	95	91
Brij®-35 (1)	99	97

** Samples (0.1 ml containing 100 µg BSA and detergent) were processed through 0.5 ml of Pierce Detergent Removal Resin and the percent detergent removed was determined. Similar results were produced for insulin (5.7 kDa), α–lactalbumin (14.2 kDa) and carbonic anhydrase (29 kDa) (data not shown).*

Detergent removal from peptide samples is a challenge, especially for MS analysis in which even low detergent concentrations contaminate instruments and interfere with column binding, elution and ionization. We used the Pierce Detergent Removal Resin to remove a variety of detergents from BSA and HeLa cell lysate tryptic digests followed by LC-MS/MS and MALDI-MS analysis (Figures 2 and 3).

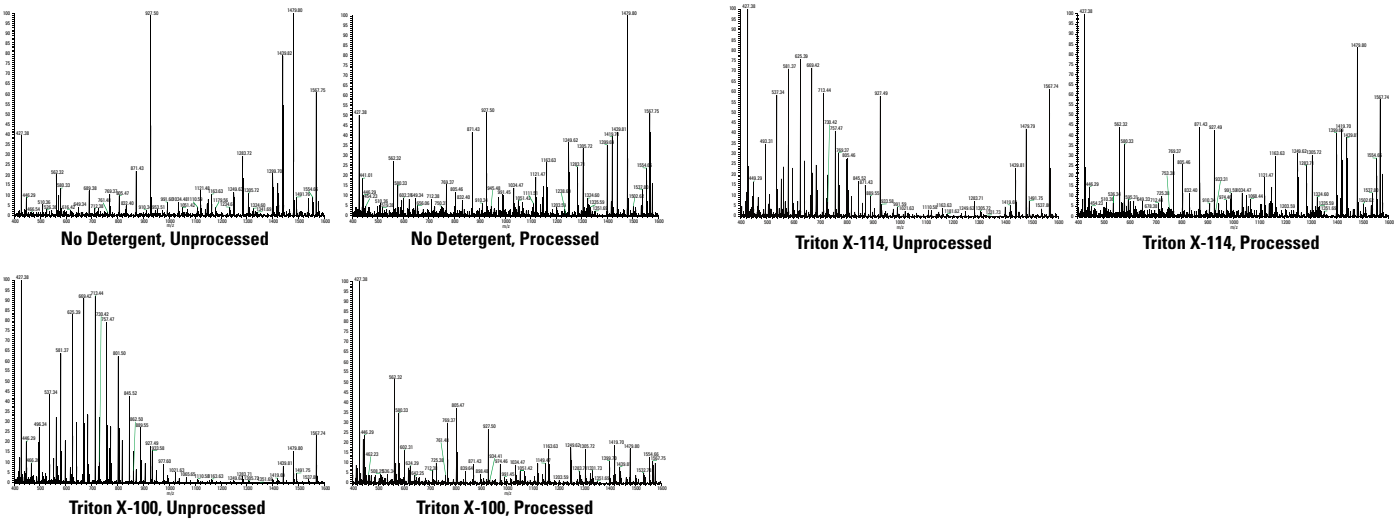


Figure 2. Peaks corresponding to detergents are eliminated in processed samples, allowing reliable peptide/protein identification. BSA tryptic digest (0.1 ml, 100 µg) containing a detergent was processed through 0.5 ml of Pierce Detergent Removal Resin and subjected to MALDI-MS analysis on a MALDI-Orbitrap Mass Spectrometer. Similar results were produced for samples containing CHAPS, NP-40 and SDS (data not shown).

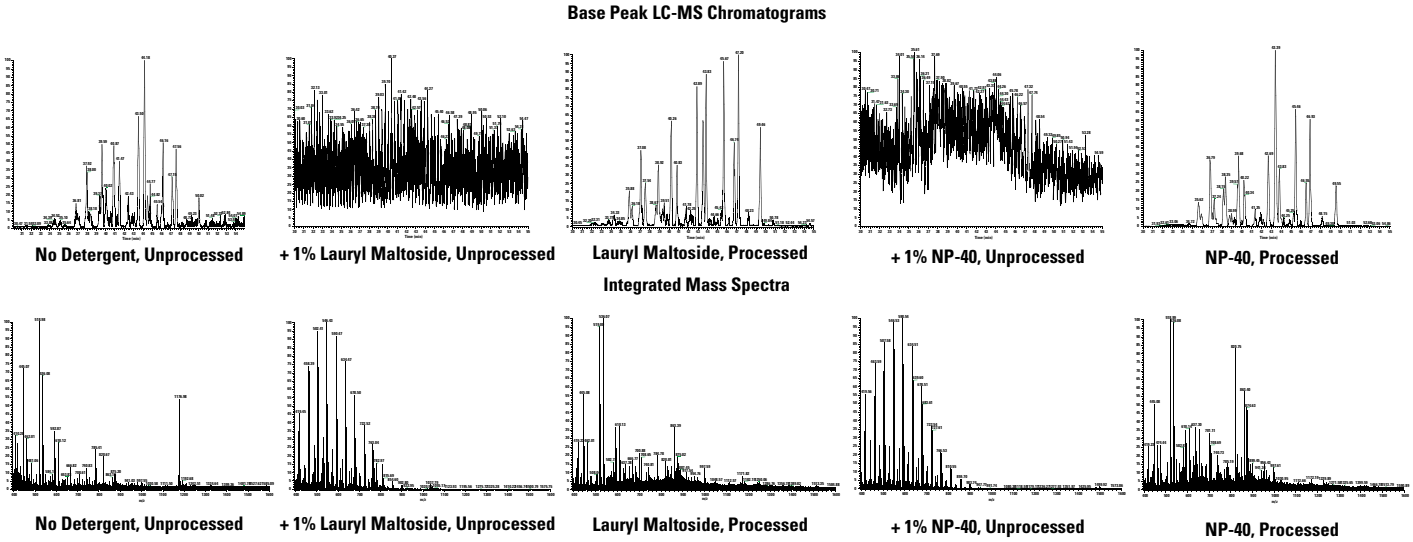


Figure 3. Effective detergent removal eliminates interference and allows high sequence coverage analysis of BSA. Tryptic digests (0.1 ml, 100 µg) containing detergent were each processed through 0.5 ml of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. **Top Row:** Base peak LC-MS chromatograms. **Bottom Row:** Integrated mass spectra. Similar results were produced for Brij-35, octyl glucoside, octyl thioglucoside and SDS (data not shown).

After processing samples, the high baseline caused by detergents is reduced or eliminated. Analysis of digested HeLa cell lysates by LC-MS/MS resulted in an approximate four-fold increase of identified peptides compared to a contaminated sample and equivalent numbers of peptides compared to a control sample, indicating minimal losses of peptides (Figure 4).

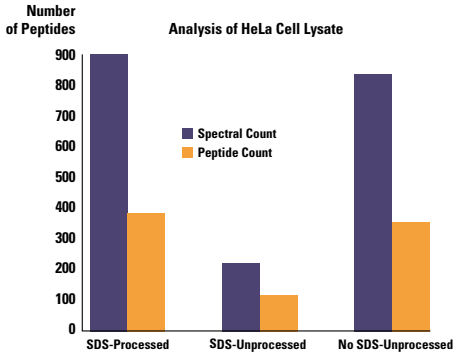


Figure 4. Effective detergent removal enables greater peptide identification. A tryptic digest of HeLa cell lysate (0.1 ml, 100 µg) containing 1% SDS was processed through 0.5 ml of Pierce Detergent Removal Resin and subjected to LC-MS/MS analysis. The processed sample allowed similar numbers of identified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

Method

Detergent removal analysis: Protein samples (1 mg/ml) containing detergent in 0.15 M NaCl and 0.05% sodium azide were processed through 0.5 ml of Pierce Detergent Removal Resin. Residual SDS was measured using Stains-All (Sigma Aldrich);[1] Triton X-100, Triton X-114 and NP-40 were measured by absorbance at 275 nm (protein absorbance was subtracted); sodium deoxycholate, CHAPS, octyl glucoside, octyl thioglucoside and lauryl maltoside were measured using concentrated sulfuric acid and phenol.[2] Removal of Brij-35 was monitored by LC-MS/MS and MALDI-MS analysis. Protein concentration was determined with the Thermo Scientific Pierce BCA Protein Assay (Product # 23225).

LC-MS/MS and MALDI-MS analysis: BSA and HeLa lysate (1 mg/ml) in 50 mM ammonium bicarbonate buffer, pH 8.0 were digested overnight with trypsin at 37°C (enzyme-to-protein ratio, 1:50) in the presence of 1% of each detergent except SDS, which was added after trypsin digestion. Each sample (0.1 ml) was processed through 0.5 ml of Pierce Detergent Removal Resin. Control samples (unprocessed) were not processed. Samples were diluted and loaded (~1.5 pmol) directly onto a C18 column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ Mass Spectrometer. For MALDI-MS analysis, samples were diluted 1:15 (1 pmol) and analyzed using a Thermo Scientific MALDI-Orbitrap Mass Spectrometer. The matrix was alpha-cyano 4-hydroxy cinnamic acid (5 mg/ml) with acetonitrile/water/0.1% TFA as a co-solvent.

References

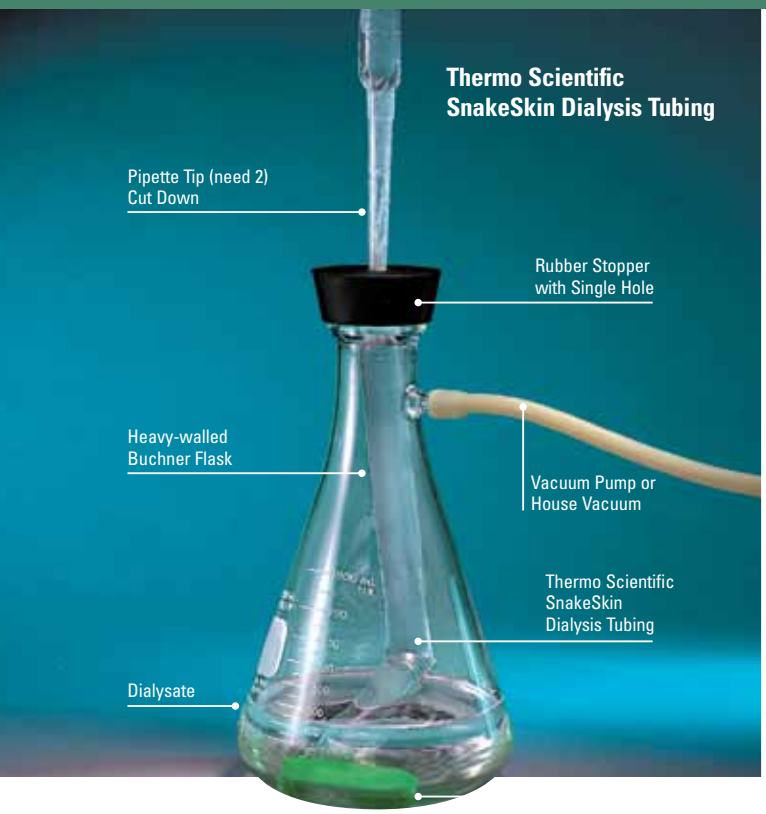
1. Rusconi, F., *et al.* (2001). Quantitation of sodium dodecyl sulfate in microliter-volume biochemical samples by visible light spectroscopy. *Anal. Biochem.* **295**:31-37.
2. Urbani, A. and Warne, T. (2005). A colorimetric determination for glycosidic and bile salt-based detergents: applications in membrane protein research. *Anal. Biochem.* **336**:117-124.

Ordering Information

Product #	Description	Pkg. Size
87776	**Pierce Detergent Removal Micro Spin Column** 125 ml settled resin/column	25 columns
87777	**Pierce Detergent Removal Spin Column, 0.5 ml** 0.5 ml settled resin/column	25 columns
87778	**Pierce Detergent Removal Spin Column, 5 ml** 2 ml settled resin/column	5 columns
87779	**Pierce Detergent Removal Spin Column, 10 ml** 4 ml settled resin/column	5 columns
87780	**Pierce Detergent Removal Resin**	10 ml

Forced Dialysis for Sample Concentration

Concentrates 100 ml down to 12 ml in six hours



Small samples dialyze much faster than larger samples because the concentration differential is much higher and the migratory diffusion distance is shorter. With a 100 ml dilute sample, it is often prudent to concentrate down to 12 ml with forced dialysis using SnakeSkin Tubing before dialysis in a Slide-A-Lyzer Dialysis Cassette. The following forced dialysis SnakeSkin Tubing application has been adapted from the method described in:

Doonan, S. (ed.) (1996). Protein Purification Protocols in *Methods in Molecular Biology,* **59**, 97-101.

Method

1) Cut off and discard the bottom of two pipette tips (2.5-5 ml) so SnakeSkin Tubing easily fits through the pipette tip.
2) Insert one pipette through the rubber stopper.
3) Cut off the desired length of SnakeSkin Dialysis Tubing (for larger volumes, the membrane will extend above the flask).
4) Thread the SnakeSkin Dialysis Tubing (dry) through the rubber stopper containing the pipette tip.
5) Clip or tie several knots in the lower end of the SnakeSkin Tubing.
6) Pour the sample to be concentrated through the top of the open end of the SnakeSkin Tubing. (Before you completely fill the SnakeSkin Tubing, place the second pipette tip inside the SnakeSkin Tubing to create a secure seal between the SnakeSkin Dialysis Tubing and the first pipette tip.) Fill with remaining sample.
7) Place 3-4 cm of buffer in the flask.
   NOTE: Most of the SnakeSkin Tubing will not be exposed to buffer.
8) Clip or tie the open end of the SnakeSkin Dialysis Tubing to ensure a closed vacuum system.
9) Connect the side arm to house vacuum.
10) Concentrate sample until desired volume is reached.

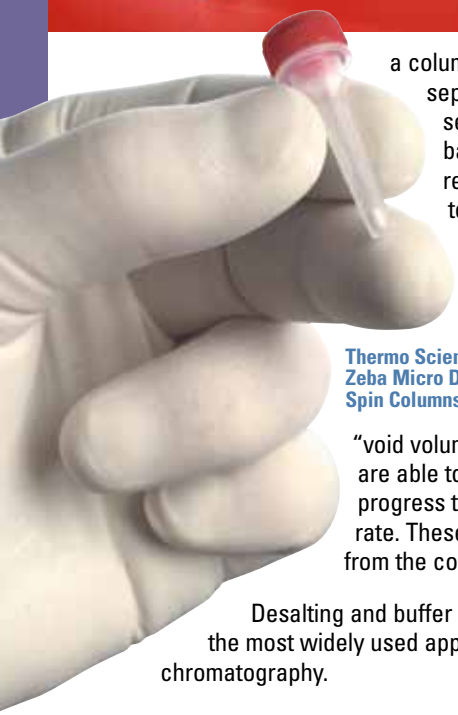
Sample Results

1) A 1 mg/ml solution of bovine serum albumin was prepared in phosphate-buffered saline, pH 7.4.
2) Approximately 30 cm of SnakeSkin Dialysis Tubing was used and assembled as described previously.
3) After six hours, the starting sample volume (100 ml) was concentrated to 12 ml with an estimated protein recovery of 65%.

Evaporation for Sample Concentration

Water inside a Slide-A-Lyzer Dialysis Cassette will evaporate. The cassette is ideally suited for sample concentration via evaporation because of the dual membranes and high surface area. Place a sample in the cassette, then withdraw the air inside. Let your sample evaporate on the bench top (using a fan to increase airflow across the membrane will speed up the process), making sure to check every 10 minutes or less to prevent evaporation to dryness. When concentrating by evaporating the water from your sample, the small molecules (buffer salts, reducing agents, etc.) will also be concentrated because no diffusion occurs.

Desalting Columns and Plates



Thermo Scientific Zeba Micro Desalt Spin Columns

Gel Filtration

Gel filtration involves the chromatographic separation of molecules of different dimensions based on their relative abilities to penetrate into a suitable stationary phase. A chromatographic resin, usually consisting of very small, uncharged porous particles in an aqueous solution, is packed into a column and then used for the separation. Different levels of separation can be achieved based on the pore size of the resin. The resin can be chosen to totally exclude proteins or large molecules, while still including small solutes. Large molecules are excluded from the internal pores of the resin and emerge first from the column in the "void volume." The smaller molecules are able to penetrate the pores, then progress through the column at a slower rate. These smaller molecules emerge from the column after the target sample.

Desalting and buffer exchange are two of the most widely used applications of gel filtration chromatography.

Desalting

Desalting involves the chromatographic separation of macromolecules in the void volume from smaller molecules that penetrate the gel bed.

Applications:

- Removing salts from protein solutions
- Removing phenol from nucleic acid preparations
- Separating excess crosslinker from conjugate preparations
- Removing excess derivatizing agents from modified proteins
- Removing unreacted dye from fluorescent antibodies
- Removing free radiolabel from labeled proteins

Buffer Exchange

Buffer exchange is used to place a protein solution into a more appropriate buffer prior to applications such as electrophoresis, ion exchange or affinity chromatography. In both desalting and buffer exchange, the macromolecular components are recovered in equilibrium with the same buffer used to equilibrate the column. If water is used for equilibration, the components will be desalted. If another buffer is used, a buffer exchange will result.

Protein Desalting Spin Columns

Thermo Scientific Zeba Desalt Spin Columns

Highlights:

- Exceptional protein recovery
- Wide product offering accommodates your sample needs
- Easy to use with no cumbersome column preparation or equilibration
- No screening fractions for protein or waiting for protein to emerge by gravity flow
- Minimal sample dilution

Although numerous techniques and resins for desalting are available, most have many drawbacks, including significant sample loss, long processing times and the need to collect multiple fractions. Zeba Desalt Spin Columns provide excellent protein recovery without the limitations associated with other desalting methods. Zeba Desalt Spin Columns are available in micro†, 0.5, 2, 5 and 10 ml formats and allow processing of samples ranging from 2 µl to 4 ml (Table 1).

Table 1. Recommended sample volumes for Thermo Scientific Zeba Spin Columns.

Resin Bed	Sample Volume
75 µl (micro) column	2-12 µl
0.5 ml column	30-130 µl
2 ml column	200-700 µl
5 ml column	600-2,000 µl
10 ml column	1,500-4,000 µl
96-well	20-100 µl

The easy-to-use Zeba Spin-Column Format dramatically improves results over standard drip-column methodologies, eliminating the need to wait for samples to emerge by gravity flow and the need to monitor fractions for protein recovery. Zeba Desalt Columns require no chromatographic system, cumbersome column preparation or equilibration and they can process multiple samples in ~8 minutes.

Zeba Desalt Spin Columns contain a high-performance desalting resin that offers exceptional desalting and protein-recovery characteristics compared to other commercially available resins (Figure 1). Samples containing as low as 25 µg/ml of protein can be processed, providing exceptional protein recovery and ≥ 95% retention of salts and other small molecules (< 1,000 MW).

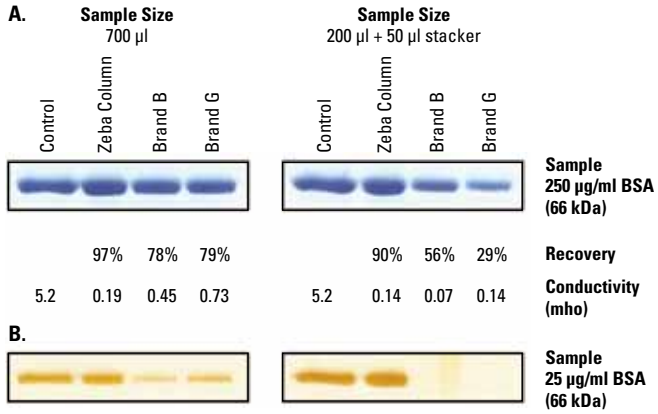


Figure 1. Increased protein recovery with Thermo Scientific Zeba Desalt Spin Columns. Samples of bovine serum albumin (BSA) at **Figure 1A.** 250 µg/ml and **Figure 1B.** 25 µg/ml in 1 M NaCl were desalted with the 2 ml Zeba Desalt Spin Columns and other commercial desalting resins using similar formats. A portion of the recovered sample (10 µl) was analyzed by SDS-PAGE. The remaining sample was used for conductivity measurements and Pierce BCA Protein Assay (Product # 23225) was performed to determine protein concentration. Zeba Desalt Resin provides significantly greater protein recovery under all conditions tested. Conductivity and protein recovery values after desalting are indicated for 250 µg/ml samples.

Desalting Columns and Plates

Ordering Information

Product #	Description	Pkg. Size
89877	Zeba Micro Desalt Spin Columns† 7K MWCO *For 5-14 µl samples.*	25/pack
89878	Zeba Micro Desalt Spin Columns† 7K MWCO *For 5-14 µl samples.*	50/pack
89882	Zeba Desalt Spin Columns, 0.5 ml 7K MWCO *For 7-200 µl samples.*	25/pack
89883	Zeba Desalt Spin Columns, 0.5 ml 7K MWCO *For 7-200 µl samples.*	50/pack
89889	Zeba Desalt Spin Columns, 2 ml 7K MWCO *For 200-900 µl samples.*	5/pack
89890	Zeba Desalt Spin Columns, 2 ml 7K MWCO *For 200-900 µl samples.*	25/pack
89891	Zeba Desalt Spin Columns, 5 ml 7K MWCO *For 300-2,000 µl samples.*	5/pack
89892	Zeba Desalt Spin Columns, 5 ml 7K MWCO *For 300-2,000 µl samples.*	25/pack
89893	Zeba Desalt Spin Columns, 10 ml 7K MWCO *For 1,000-4,000 µl samples.*	5/pack
89894	Zeba Desalt Spin Columns, 10 ml 7K MWCO *For 1,000-4,000 µl samples.*	25/pack

Ordering Information

Product #	Description	Pkg. Size
87764	Zeba Micro Desalt Spin Columns 40K MWCO *For 5-14 µl samples.*	25 columns
87765	Zeba Micro Desalt Spin Columns 40K MWCO *For 5-14 µl samples.*	50 columns
87766	Zeba Desalt Spin Columns, 0.5 ml 40K MWCO *For 7-200 µl samples.*	25 columns
87767	Zeba Desalt Spin Columns, 0.5 ml 40K MWCO *For 7-200 µl samples.*	50 columns
87768	Zeba Desalt Spin Columns, 2 ml 40K MWCO *For 200-900 µl samples.*	5 columns
87769	Zeba Desalt Spin Columns, 2 ml 40K MWCO *For 200-900 µl samples.*	25 columns
87770	Zeba Desalt Spin Column, 5 ml 40K MWCO *For 300-2,000 µl samples.*	5 columns
87771	Zeba Desalt Spin Column, 5 ml 40K MWCO *For 300-2,000 µl samples.*	25 columns
87772	Zeba Desalt Spin Column, 10 ml 40K MWCO *For 1,000-4,000 µl samples.*	5 columns
87773	Zeba Desalt Spin Column, 10 ml 40K MWCO *For 1,000-4,000 µl samples.*	25 columns

Thermo Scientific Pierce Spin Columns (No Resin)

Ordering Information

Product #	Description	Pkg. Size
89879	Pierce Micro Spin Columns	50/pack
89868	Pierce Spin Columns, 0.5 ml	25/pack
89896	Pierce Spin Columns, 2 ml	25/pack
89897	Pierce Spin Columns, 5 ml	25/pack
89898	Pierce Spin Columns, 10 ml	25/pack

Thermo Scientific Zeba 96-Well Desalt Spin Plates

Highlights:

- Desalt protein in one fraction with no dilution
- Exceptional protein recovery
- Easy to use with no cumbersome plate preparation or equilibration
- Minimal sample dilution

The new Thermo Scientific Zeba 96-Well Desalt Spin Plates provide high-throughput removal of salt and small molecules from samples, preparing them for downstream analysis, including mass spectrometry, HPLC, capillary electrophoresis, metabolite screening and assay development.

The Zeba 96-Well Desalt Spin Plates contain a high-performance resin that provides exceptional desalting and protein recovery characteristics. Process small (20-100 µl) sample volumes and achieve exceptional protein recovery (Table 1) and > 95% removal of salts and other small molecules (< 1,000 Da) such as DTT, biotin, FITC or biotin-FITC. The Zeba 96-Well Desalt Spin Plates require no resin dispensing or hydration. One plate of 96 samples can be processed in 5 minutes.

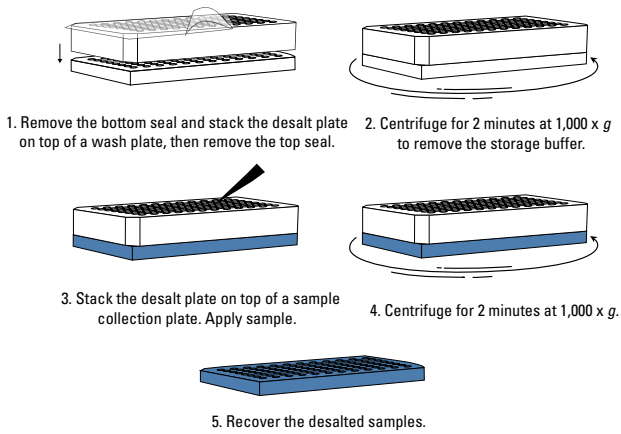


Figure 1. Thermo Scientific Zeba 96-Well Desalt Spin Plates are easy to use.

Table 1. Protein recovery and desalting efficiency.

Protein	% Protein Recovery	% NaCl Removed
BSA (66 kDa)	98.5	> 95
α-Lactalbumin (14.1 kDa)	91.5	> 95
Ubiquitin (8.6 kDa)	85	> 95

Protein samples (1 mg/ml) were prepared in 1.0 M NaCl and 100 µl samples were desalted using Zeba Desalt Spin Plates. Results were analyzed by Pierce BCA Protein Assay and conductivity measurements.

Ordering Information

Product #	Description	Pkg. Size
89807	**Zeba 96-well Desalt Spin Plates** Each well contains ~550 µl resin slurry and can process 20-100 µl samples. The package contains two wash plates and two collection plates.	2 plates
89808	**Zeba 96-well Desalt Spin Plates** Each well contains ~550 µl resin slurry and can process 20-100 µl samples. The package contains two wash plates and four collection plates.	4 plates
89934	**Pierce Desalting Chromatography Cartridges** Each cartridge is packed with 1 ml Zeba Desalting Resin. Recommended for processing compounds > 7K MW.	5 x 1 ml
89935	**Pierce Desalting Chromatography Cartridges** Each cartridge is packed with 5 ml Zeba Desalting Resin. Recommended for processing compounds > 7K MW.	5 x 5 ml

Protein Concentrators

Protein Concentration

Protein concentration is a commonly performed and essential procedure for sample preparations. There are a variety of devices available, all containing ultrafiltration membranes with a range of molecular-weight cutoffs (MWCO). The type of device used can significantly affect protein recovery, especially with low-concentration samples that are often lost or damaged in the process.

Thermo Scientific Pierce Protein Concentrators

The new Thermo Scientific Pierce Concentrators are disposable ultrafiltration centrifugal devices for concentration and diafiltration/buffer-exchange of biological samples such as enzymes, antigens or antibodies. Because of their unique design, the Pierce Concentrators avoid the problems commonly associated with protein concentration. These concentrators consist of a high-performance regenerated cellulose membrane welded to a conical device and are compatible with swinging-bucket and fixed-angle rotors. The design enables a high degree of concentration in a single centrifugation step, while minimizing polarization and adsorption at the membrane surface. Additionally, researchers can accurately control the dead-stop volume and final concentration factor for reliable and consistent sample processing. Concentration factors of > 110-fold are achieved in 45 minutes with the 20 ml devices (Table 1). The membranes are accurately rated and routinely provide > 90% recovery of proteins larger than the membrane MWCO, and > 85% with low-concentration samples (Tables 1 and 2).

Highlights:

- **Superior protein concentration and recovery** – achieve > 110-fold protein concentration in 45 minutes with > 90% protein recovery
- **Convenient** – concentrate 1-20 ml of sample in a fast spin format
- **Instrument-compatible** – use swinging-bucket or fixed-angle rotors; collect sample without invert spinning
- **Versatile** – the 150K MWCO concentrator is ideal for samples containing microorganisms such as viral particles

Table 1. Thermo Scientific Pierce Concentrators provide exceptional recovery with low-concentration samples.

Pierce Concentrator	Protein†	Time (min)	Protein Concentration (mg/ml)	Recovery (%)	Fold Concentration‡
20 ml/9K*	Cytochrome c	45	0.2	100	121
			0.01	96	117
20 ml/20K**	BSA	45	0.2	98	137
			0.01	95	118
7 ml/9K*	Cytochrome c	35	0.2	96	137
			0.01	88	129
7 ml/20K**	BSA	35	0.2	97	81
			0.01	87	65

†Protein samples were centrifuged at 3,000 x g for 45 minutes at 22°C using a starting volume of 20 ml with the 20 ml concentrators and 5 ml with the 7 ml concentrators.
‡Fold concentration was determined by dividing the starting volume by the recovered (retentate) volume.
**For the 9K concentrators, percent recovery was calculated by measuring the absorbance at 409 nm of the retentate adjusted with buffer to the original volume.*
***For the 20K concentrators, percent recovery was determined using the Thermo Scientific Micro BCA Protein Assay (Product # 23235).*

Table 2. Typical retention of proteins using the 150 MWCO concentrator.*

Protein	Concentration (mg/ml)	Recovery (%)
IgG (150 kDa)	0.5	93
Aldolase (160 kDa)	0.25	87

** Protein solutions (13.5 ml) in phosphate-buffered saline were concentrated in a fixed-angle rotor at 2,000 x g at 22°C. The concentration was determined by Pierce BCA Protein Assay.*

Pierce Concentrators effectively combine speed, capacity and recovery for high performance concentration, purification and separation of proteins even with dilute samples. We compared the Pierce Concentrators to ultrafiltration centrifugal devices from the other suppliers. Using a variety of test proteins and starting concentrations, protein recovery was monitored by Thermo Scientific Pierce BCA Protein Assay (Product #23225). For samples above the rated MWCO, high levels of protein was recovered (approximately 90% or greater) with Pierce Concentrators with a starting concentration as low as 0.02 mg/ml (Table 3). Significantly lower or no protein was recovered with devices from supplier V, indicating inappropriate molecular weight ratings or significant binding of protein to the device membrane.

Applications:

- Protein concentration with tissue culture media, antiserum or monoclonal antibody preparations
- Concentration of protein peaks following gel-permeation chromatography
- Removal of unincorporated protein label
- Concentration and desalt/buffer-exchange after eluting protein from ion-exchange, hydrophobic interaction (HIC), metal-chelate or affinity-chromatography columns

Table 3. Thermo Scientific Pierce Concentrators perform better than units from other suppliers.*

Concentrator (MWCO)	Recovery (%)		
	BSA (66,000 MW)	Lysozyme (14,000 MW)	Ubiquitin (8,700 MW)
Pierce Concentrator (9K)	91	98	96
Supplier M (10K)	90	93	91
Supplier V (10K)	76	5	5

** Proteins samples (~0.02 mg/ml) were centrifuged in concentrators at 3,000 x g until a 15- to 25-fold decrease in sample volume was achieved. Samples were recovered without membrane washing. Recovery was determined using the Pierce BCA Protein Assay. Results were unaffected by centrifugation rate and similar for the 7 and 20 ml Pierce Concentrators. Similar or higher recovery values were obtained with the Pierce Concentrators at higher protein loads (data not shown).*

Ordering Information

Product #	Description	Pkg. Size
87748	Pierce Concentrators, 9K/7 ml	10/pkg.
89884A	Pierce Concentrators, 9K/7 ml	25/pkg.
87749	Pierce Concentrators, 9K/20 ml	10/pkg.
89885A	Pierce Concentrators, 9K/20 ml	25/pkg.
87750	Pierce Concentrators, 20K/7 ml	10/pkg.
89886A	Pierce Concentrators, 20K/7 ml	25/pkg.
87751	Pierce Concentrators, 20K/20 ml	10/pkg.
89887A	Pierce Concentrators, 20K/20 ml	25/pkg.
89920	Pierce Concentrators, 150K/7 ml	10/pkg.
89922	Pierce Concentrators, 150K/7 ml	25/pkg.
89921	Pierce Concentrators, 150K/20 ml	10/pkg.
89923	Pierce Concentrators, 150K/20 ml	25/pkg.

Thermo Scientific Pierce Cell Lysis Reagents Selection Guide

Description	Organisms/Samples	Dialyze[1]	Compatibility
B-PER Reagent[†] **78243, 165 ml 78248, 500 ml**	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α >M15, Archaebacteria, nematodes and *Acinetobacter* sp.	Yes	Reporter assays, IPs[2], Western blot, GST- and histidine-tag purification
B-PER II Reagent 78260, 250 ml (A 2X version of B-PER Reagent)	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.	Yes	Reporter assays, IPs[2], Western blot, GST- and histidine-tag purification
B-PER PBS Reagent 78266, 500 ml	Gram(-) bacteria, *S. aureus, H. pylori, E. coli* strains BL21(D3)> JM109> DH5α>M15, Archaebacteria, nematodes and *Acinetobacter* sp.	Yes	Reporter assays, IPs[2], Western blot, GST- and histidine-tag purification
Y-PER Reagent 78991, 200 ml 78990, 500 ml	*S. cerevisiae, Schizo-saccharomyces pombe, C. albicans, B. subtilis, E. coli, P. pastoris, Strep. avidinii* and *Acinetobacter* sp.	No	IPs[2], Western blot, β-Gal enzyme assays, IEF after dialysis, GST- and histidine-tag purification
Y-PER Plus Reagent 78998, 25 ml 78999, 500 ml	Yeast (*S. cerevisiae*) and *Acinetobacter* sp.	Yes	GST- and histidine-tag purification, Western blot
M-PER Reagent 78503, 25 ml 78501, 250 ml 78505, 1 L	Cultured mammalian cells, COS-7, NIH 3T3, Hepa 1-6, 293, CHO, MDA, MB231 and FM2	Yes	Luciferase, β-Gal (low signal), CAT, kinase assays, ELISAs, immobilized glutathione, Western blot
P-PER Plant Protein Extraction Reagent 89803, Kit	Multiple plant organs (leaf, stem, root, seed and flowers); multiple plant species (*Arabidopsis*, tobacco, maize, soybeans, peas, spinach, rice and other plant tissues); and fresh, frozen and dehydrated plant tissues	No	1-D and 2-D gel electrophoresis, Western blotting, activity assays and protein affinity purifications*
T-PER Reagent 78510, 500 ml	Heart, liver, kidney and brain	Yes	Luciferase, β-Gal, CAT, kinase assays, Western blot, ELISAs, immobilized glutathione
I-PER Reagent 89802, 250 ml	Baculovirus-infected insect cells grown in suspension or monolayer culture	No	Western blot, 6xHis-tagged protein purification, protein assays and ion-exchange chromatography
NE-PER Reagent 78833	Tissue: calf liver. Tissue: mouse heart, kidney, lung and liver; Cultured cells: epithelial (HeLa), fibroid (COS-7), kidney (NIH 3T3), liver (Hepa 1) and brain (C6)	No (CER) Yes (NER)	EMSA (if using < 3 µl or 10%, otherwise dialyze first in SAL MINIs[5]), Western blot, reporter assays, IEF (after dialysis to reduce salt concentration) and 2-D[6]
Mem-PER Reagent 89826	Cultured cells: brain (C6), epithelial (HeLa), fibroblasts (NIH 3T3) and yeast (*S. cerevisiae*)	Yes[4]	Western blot and 2-D[6]
Subcellular Protein Fractionation Kit 78840	Cultured mammalian cells	Yes	Protein assays, Western blotting, gel-shift assays and enzyme activity assays
Mitochondria Isolation Kit for Cultured Cells[†] **89874**	Mammalian cells	Yes[7]	Western blot, 2-D Western blots, electrophoresis. Applications include apoptosis, signal transduction and metabolic studies.
Mitochondria Isolation Kit for Tissue[†] **89801**	Heart, liver, kidney and brain		
Pierce RIPA Buffer 89900, 100 ml 89901, 250 ml	Cultured mammalian cells and cytoplasmic, membrane and nuclear proteins	Yes	Reporter assays, protein assays, immunoassays and protein purification
Lysosome Enrichment Kit for Tissues and Cultured Cells 89839	Tissues and cultured cells	N/A	2-D/MS, electron microscopy, disease profiling, gene expression, signal transduction, and interaction or localization studies
Peroxisome Enrichment Kit for Tissue 89840	Heart, liver, kidney and brain	N/A	2-D/MS, electron microscopy, disease profiling, gene expression, signal transduction, and interaction or localization studies
Pierce IP Lysis Buffer 87787, 100 ml 87788, 250 ml	Cultured mammalian cells	Yes	Immunoprecipitation, pull-downs, Co-IP, protein assays, Western blotting
Nuclei Enrichment Kit for Tissue 89841	Heart, liver, kidney and brain	N/A	2-D/MS, electron microscopy, disease profiling, gene expression, signal transduction, and interaction or localization studies

1. The detergent can be removed by dialysis
2. Immunoprecipitation
3. Halt Protease Inhibitor Cocktail, Product #s 78425 (EDTA-free) and 78430
4. Samples prepared in Mem-PER Reagent can be dialyzed if the buffer contains detergent (e.g., CHAPS), otherwise use Pierce SDS-PAGE Sample Prep Kit (Product # 89888)
5. Slide-A-Lyzer MINI Dialysis Units
6. 2-D Sample Prep for Nuclear Proteins (Product # 89863) and 2-D Sample Prep for Membrane Proteins (Product # 89864) were designed using our NE-PER and Mem-PER Reagents.
7. Need to lyse mitochondria first.

Protein Assay Compatibility	Notes
Pierce BCA Assay and Coomassie Plus Assay	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents and reducing agents can be added for more efficient lysis. Do not exceed 0.5 M NaCl. Better lysis if cells are frozen in B-PER Reagent.
Pierce BCA Assay and Coomassie Plus Assay after Compat-Able™ Protein Assay Reagent Set (Product # 23215) or dilute two to four times; Pierce 660 nm Protein Assay after 2-fold dilution	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents and reducing agents can be added for more efficient lysis. Better lysis if cells are frozen in B-PER Reagent.
Pierce BCA Assay and Coomassie Plus Assay after Compat-Able Protein Assay Reagent Set (Product # 23215) or dilute two to four times	Protease inhibitors[3] may be added to prevent protein degradation. Salts, chelating agents and reducing agents can be added for more efficient lysis. Better lysis if cells are frozen in B-PER Reagent.
Pierce BCA Assay	Protease inhibitors[3] may be added to prevent protein degradation. Use at room temperature. Double incubation time for use at 4°C. Use log-phase cells. For stationary phase cells, add 0.1 M DTT or 20-50 mM TCEP. Will work with 1 mM EDTA. Does not lyse spores. Cannot use with ion exchange columns.
Pierce BCA Assay and Coomassie Plus Assay	Protease inhibitors[3] may be added to prevent protein degradation. The addition of up to 2 M NaCl may result in increased efficiency of lysis and protein yield.
Pierce BCA Assay and Coomassie Plus Assay; Pierce 660 nm Protein Assay after 2-fold dilution	Protease inhibitors[3] may be added to prevent protein degradation. Adding 150 mM NaCl results in increased efficiency of lysis and higher protein yield in some cells lines. A PBS rinse of cells prior to lysis removes contaminants such as phenol red and increases protein yield.
Pierce BCA Assay, Reducing Agent-Compatible; Not compatible with Bradford, Coomassie or the original Pierce BCA Assay; Pierce 660 nm Protein Assay after 2-fold dilution	Kit lyses most plant cells without harsh mechanical lysis aids; extremely fibrous tissues such as woody stems may require mechanical grinding by devices not included in this kit. P-PER Extracts can be quantified using the Pierce BCA Protein Assay Kit, Reducing Agent Compatible (Product # 23250).
Pierce BCA Assay (dilute 1:1) and Coomassie Plus Assay; Pierce 660 nm Protein Assay after 2-fold dilution	Protease inhibitors[3] may be added to prevent protein degradation. Mechanical disruption of the tissue is still required. Can also be used for cultured cells.
Pierce BCA Assay	Protease inhibitors[3] may be added to prevent protein degradation.
Pierce BCA Assay and Coomassie Plus Assay (dilute CER Reagent mixture four times); Pierce 660 nm Protein Assay after 2-fold dilution	Protease inhibitors[3] may be added to prevent protein degradation. Packed cell vol.: 2 x 10^6 HeLa cells = 10 µl = 20 mg. Tissue yield (calf liver): 3-4 mg cytoplasmic protein/100 mg tissue; 1-1.5 mg nuclear protein/100 mg tissue. Cell yield (HeLa): 300-400 µg cytoplasmic protein/10^6 cells; 40-60 µg nuclear protein/10^6 cells. Positive controls tested: cytoplasmic (β-Gal, PKC, Hsp90); nuclear (Oct-1, p53, DNA polymerase).
Pierce BCA Assay and Coomassie Plus Assay; hydrophobic phase needs to be dialyzed first; see instruction book; Pierce 660 nm Protein Assay after 2-fold dilution	Protease inhibitors[3] may be added to prevent protein degradation. Can dialyze against another detergent (e.g., CHAPS). Extraction efficiency is generally > 50% with the cell lines tested (having proteins with up to two transmembrane segments).
Pierce BCA Assay; Pierce 660 nm Protein Assay	Kit includes protease inhibitors to prevent proteolysis and phosphatase inhibitors that may be added to maintain phosphorylation of proteins.
Pierce BCA Assay (after lysis)	Protease inhibitors may be added to prevent protein degradation. Douncing will increase isolation efficiency vs. detergent alone; however, multiple samples can be processed simultaneously using the reagent-based methods.
Pierce BCA Assay	Protease inhibitors[3] may be added to prevent proteolysis and phosphatase inhibitors may be added to maintain phosphorylation of proteins.
Coomassie Plus – The Better Bradford Assay Kit	Protease inhibitors[3] may be added to prevent proteolysis and phosphatase inhibitors may be added to maintain phosphorylation of proteins.
Coomassie Plus – The Better Bradford Assay Kit	Protease inhibitors[3] may be added to prevent proteolysis and phosphatase inhibitors may be added to maintain phosphorylation of proteins.
Pierce BCA Assay; Pierce 660 nm Protein Assay	Kit includes protease inhibitors to prevent proteolysis and phosphatase inhibitors that may be added to maintain phosphorylation of proteins
Coomassie Plus – The Better Bradford Assay Kit	Protease inhibitors[3] may be added to prevent proteolysis and phosphatase inhibitors may be added to maintain phosphorylation of proteins.

* Although kit works without liquid nitrogen/freeze-grinding, Dounce homogenization, blade-shearing or glass-bead agitation for cell disruption, it is compatible with these alternative mechanical aids

† See patent information on inside front cover.

Contact Information

Belgium and Europe,
the Middle East
and Africa Distributors
Tel: +32 53 85 71 84

France
Tel: 0 800 50 82 15

The Netherlands
Tel: 076 50 31 880

Germany
Tel: 0228 9125650

United Kingdom
Tel: 0800 252 185

Switzerland
Tel: 0800 56 31 40

Email: perbio.euromarketing@thermofisher.com
www.thermo.com/perbio

United States
Tel: 815-968-0747 or 800-874-3723
Customer Assistance E-mail:
Pierce.CS@thermofisher.com
www.thermo.com

1601823 12/09 Printed in U.S.

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