Spectra/Por®
MicroDialyzer

SPECTRUM PRODUCT INSTRUCTION BOOK

SPECTRUM®LABS.COM

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Introduction

Spectra/Por® dialysis membranes are the industry leader in terms of dialysis time and reproducibility. For the dialysis of small samples, where the use of bulk dialysis tubing may be impractical, Spectrum provides a variety of MicroDialyzers. Each MicroDialyzer is capable of dialyzing samples to equilibrium within two hours. Spectrum MicroDialyzers are easy to set up, so that they minimize experiment preparation time as well as dialysis time.

The Spectra/Por® MicroDialyzers are capable of simultaneously dialyzing 1 to 96 samples against a common buffer. Sample sizes can range from 24 µl to 620 µl. The use of specially framed Spectra/Por® CE membranes minimizes dialysis time while maximizing recovery.

Features

- Continuous buffer exchange maximizes the dialysis speed.
- Compatible with multichannel pipetters for quick and precise sample handling.
- Pre-framed Spectra/Por CE membranes for high dialysis rate and maximum sample recovery.
- Small membrane surface area minimizes dilution and sample loss.
- Wells are labeled for sample identification.
- Easy-to-use clamps prevent leakage.
- Detachable bottom plate for ease of cleaning.

Applications

- Sample purification prior to HPLC or electrophoresis.
- Desalting macromolecules.
- Sterile multiple microdialysis.
- Quantitative removal of electrolytes from biological fluids.
- Changing buffers and/or pH.
- Removing contaminants (cross-linkers, CsCl).
- Removal of sucrose following a sucrose density gradient.
- Binding studies of proteins and substrates.
- Concentration of antibodies.
- Sample concentration (by dialysis against glycerol).
- Dialysis of toxic or radioactive samples.
- Removal of detergents.

Dialysis

Dialysis is a means of separating molecules across a semipermeable membrane. The driving force for the separation is a concentration difference between the solutions separated by the membrane. Solutes larger than the pore size of the membrane will be retained on one side of the membrane, while solvent and small solutes will be able to pass through it.

The molecular weight cut off (MWCO) of a membrane is the molecular weight of the smallest solute retained by the membrane to an extent of 90% during a 17-hour test. Solutes of molecular weight less than the MWCO will be retained, in general, less than 90% during a 17-hour dialysis. The size of many molecules will, however, vary with pH and ionic strength so that the listed membrane MWCO's should be regarded as approximate. The user may need to test several different membranes for a particular use.

Note: Capacity of sample wells is inversely proportional to the sample concentration; i.e., if 125 µl of a concentrated sample is placed into a 150 µl well, the concentration gradient will generate a flow into the sample well, increasing the volume of the sample.

Rate of Dialysis

Dialysis membranes may also be characterized by the rate at which a permeable species passes through it. A rate test may be carried out by placing a solution of a permeable species on one side of the membrane and pure solvent on the other. If both sides are well stirred and the pure solvent is changed frequently enough so that it never contains a significant amount of solute, a first order rate for the dialysis will be observed.

Spectra/Por MicroDialyzers are designed to allow first-order kinetics, the most rapid transfer possible, to be easily attained. The diffusate chamber (the pure buffer or pure solvent chamber) is fitted with tubing connectors to allow for continuous fluid replacement; it is also supplied with a stir bar to allow the diffusate to be well stirred whether or not it is continually replaced.

Another important variable in the rate of dialysis is the molecular weight of the diffusing species. As the molecular weight of a molecule increases, its rate of dialysis decreases. When the molecular weight is grossly different from the MWCO of the membrane this is caused by a reduction in the bulk diffusion coefficient with the increase in mass. As the molecular size nears the pore size of the membrane, however, the rate of dialysis will decrease rapidly with increasing molecular weight due to the inability of the molecules to physically traverse the pores in the membrane.

Membrane MWCO Selection

The effective size of many solute molecules is affected by the pH and ionic strength of the solution. Therefore the MWCO of a membrane should be regarded as an approximate and not absolute number. To establish the best MWCO for an application it may be necessary to evaluate several membranes. To minimize the dialysis time, the maximum MWCO which does not cause undue loss of the desired species should be used.

Although the MWCO of the Spectra/Por CE membranes used with the MicroDialyzers is very sharp, separation of two species of nearly identical weight is usually not a practical pursuit with dialysis. For reasonably efficient separation the molecular weights of any two species to be separated should differ by at least a factor of twenty.

Membrane Composition

The framed Spectra/Por CE membranes for the MicroDialyzer are made of virgin synthetic cellulose acetate. This material is processed, extruded in sheet form, and then bound to the MicroDialyzer frames. These membranes contain no heavy metals or sulfides and are the most chemically pure and inert membranes available. No pretreatment prior to use is required.

Membrane Handling

Like all cellulose esters, Spectra/Por CE membranes are soluble in certain organic solvents and are swollen by others. As a consequence it is recommended that CE membranes not be exposed to solutions of organic solvents exceeding 5% concentration in water. Temperatures up to 37 °C may be employed in routine use, but higher temperatures may alter the MWCO and are not recommended.

Spectra/Por CE membranes manufactured for the Spectra/Por MicroDialyzer are very thin and must be handled with care. The extreme thinness helps to maximize the rate of dialysis.

Instruction For Use

Membrane Preparation

CAUTION: The Spectra/Por CE membranes used with the Spectra/Por MicroDialyzers are packaged in 0.1% sodium azide. Sodium azide is an acute poison and is also a skin and eye irritant. Wear gloves and eye protection while opening the membrane package and handling the membrane frame until the sodium azide has been washed away. Observe all Federal, State and local regulations when storing or disposing of sodium azide. For assistance contact the District Director of the Environmental Protection Agency.

1. While wearing gloves and eye protection, carefully open the membrane package.
2. Hold the membrane by the frame and rinse both sides with distilled water. Soak the membrane in distilled water for approximately 10 minutes to allow any sodium azide in the membrane to diffuse out.

The 5, 10, 12, and 16 well dialyzers are held together with two snap clamps. Instructions for using these dialyzers are provided here. The 96-well dialyzer is held together with 8 thumb-screws; its use is described in the next section.

CAUTION: Keep fingers clear of the snap clamp catching area.

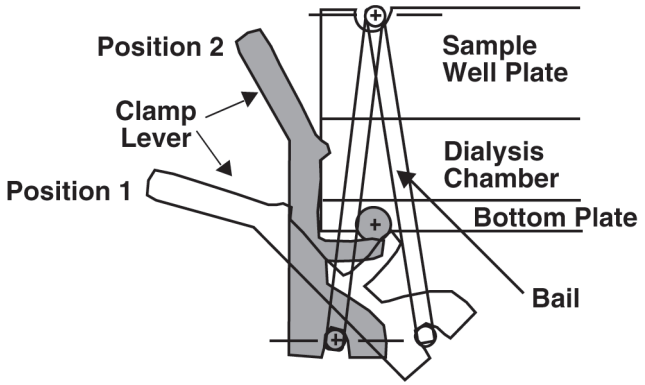

Figure 1 - Spectra/Por® MicroDialyzer Snap Clamp

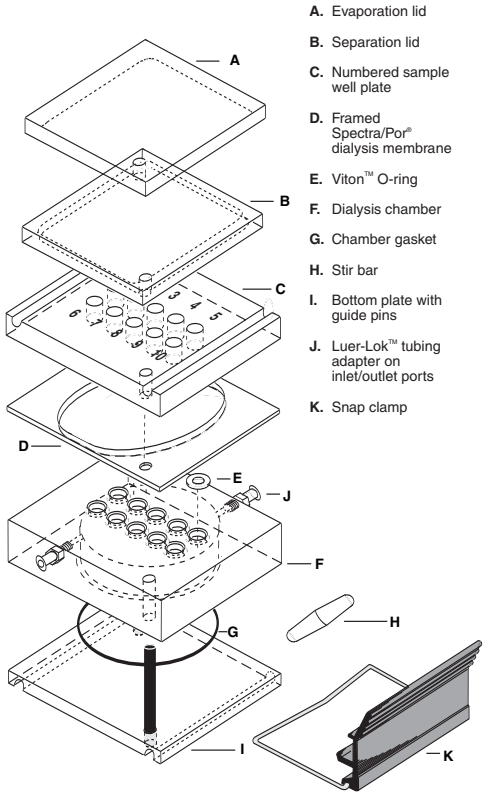

Figure 2 - Spectra/Por® MicroDialyzer components

Instruction of Use - MicroDialyzer

1. Lift off the evaporation lid.
2. Release the snap clamps. Pull the clamp lever away from the MicroDialyzer body to position 1 (see Figure 1). Then disengage the clamp from the MicroDialyzer. Remove one clamp at a time.
3. Remove the sample well plate (item B in Figure 2). The O-rings should remain on top of the dialysis chamber. If necessary, reposition any O-rings which may have been disturbed so that they are centered on top of the well openings in the dialysis chamber.
4. Position a framed Spectra/Por CE dialysis membrane on top of the O-rings (being careful not to disturb them). The holes in the membrane's frame should surround the guide posts. The shiny side of membrane should face away from the O-rings.
5. Replace the sample well plate on top of the membrane. The guide posts should slide through the mating holes machined into this plate.
6. To snap the clamps back into position, arrange the MicroDialyzer and one of the clamps as shown in Figure 1 position 1. To do this, slip the MicroDialyzer through the bail and place the bail into the groove on the sample well plate. Slide the notch on the clamp lever into the groove on the underside of the bottom plate.
7. Snap the clamp closed by pressing the lever toward the well plate.
8. Close the other snap clamp in a similar fashion.
9. Visually inspect the cell to ensure that the cell parts are properly clamped and aligned.
10. The unit is ready to be filled with dialysate. Holding the MicroDialyzer on its edge, so that one fill port is on the bottom and the other on the top, fill the buffer chamber using either a pump or a pipette. No air bubbles should be present in the buffer chamber. The presence of air bubbles will degrade the dialysis efficiency. It is recommended that the dialysate be degassed prior to use (see below).
11. Place the dialyzer in its normal operating position (with the sample wells opening upward) on a SilentSwirl® Stir Plate.
12. Fill the wells with sample, activate the stirrer, and begin pumping dialysate through the buffer chamber. A flow rate of 3 to 10 ml/min has been found to provide a sufficient rate of exchange of buffer.

If the samples are sufficiently small the MicroDialyzer may be operated without a pump, provided that the inlet and outlet ports of the buffer chamber are sealed after it has been filled with dialysate as described in step 11 above.

Use of Dialyzer Equipped with Thumb-Screw (96-well)

1. Lift off the evaporation lid.
2. Unscrew and remove the 8 thumb-screws.
3. Remove the sample well plate (item B in Figure 3). Position a membrane gasket over the alignment pins so that the holes in the gasket line up with holes in the dialysis chamber plate.
4. Position a framed Spectra/Por CE dialysis membrane on top of the membrane gasket. The holes in the membrane's frame should surround the guide posts.

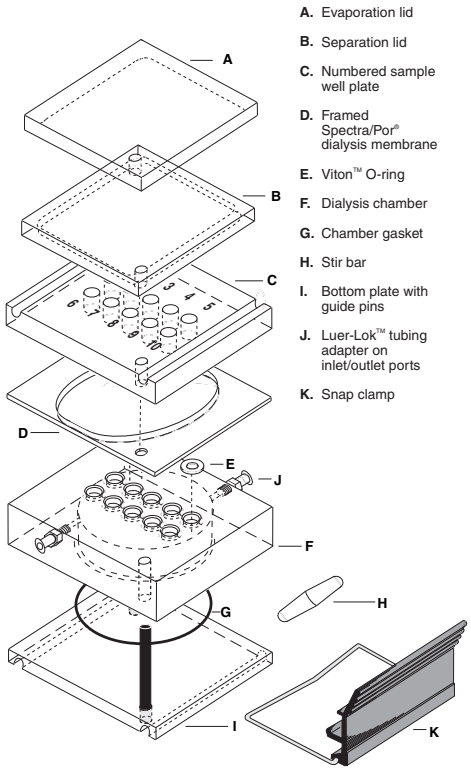

Figure 3 - Typical Thumb-screw MicroDialyzer

5. Replace the sample well plate on top of the membrane. The guide posts should slide through the mating holes machined into this plate.
6. Place all 8 thumb-screws into the MicroDialyzer and, using the sequence indicated in Figure 4, tighten them finger-tight.
7. After tightening all 8 thumb-screws, retightened them, again using the sequence indicated in Figure 4
8. Visually inspect the cell to ensure that the cell parts are properly clamped and aligned.
9. The unit is ready to be filled with dialysate. Holding the MicroDialyzer on its edge, so that one fill port is on the bottom and the other on the top, fill the buffer chamber using either a pump or a pipette. No air bubbles should be present in the buffer chamber. The presence of air bubbles will degrade the dialysis efficiency. It is recommended that the dialysate be degassed prior to use (see below).
10. Place the dialyzer in its normal operating position (with the sample wells opening upward) on a SilentSwirl Stir Plate.
11. Fill the wells with sample, activate the stirrer, and begin pumping dialysate through the buffer chamber. A flow rate of 3 to 10 ml/min has been found to provide a sufficient rate of exchange of buffer.

If the samples are sufficiently small the MicroDialyzer may be operated without a pump, provided that the inlet and outlet ports of the buffer chambers are sealed after it has been filled with dialysate as described in step 11 above.

When different types of dialysis buffers are to be used with the unit, it is recommended to replace the gasket with each buffer change.

NOTE: Capacity of sample wells is inversely proportional to the sample concentration; i.e., if 125 µl of a concentrated sample is placed into a 150 µl well, the concentration gradient will generate a flow

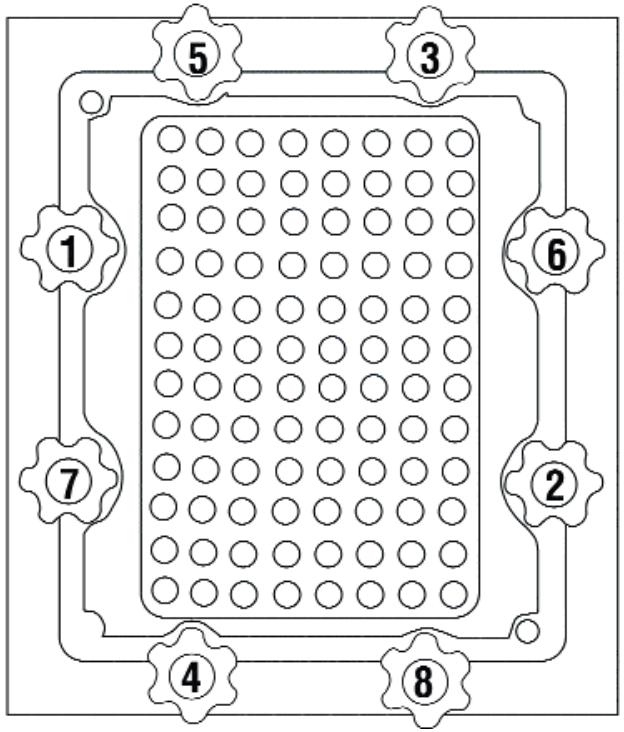

Figure 4 - 96-well bold tightening sequence

into the sample well, increasing the volume of the sample.

Degassing the Dialysate

The solubility of air in water decreases with increasing temperature, so that bubbles of air may spontaneously form with changes in the temperature of the buffer. Since the MicroDialyzer uses a horizontal membrane, these air bubbles may become trapped underneath it, reducing the dialysis efficiency.

To prevent the spontaneous formation of air bubbles it is recommended that the buffer solution be degassed immediately prior to use. The recommended method for degassing the solution is to vacuum filter it using a Spectrum Microfiltration System equipped with a 0.2 µm filter.

Cleaning MicroDialyzers

The following procedure may be used to clean the MicroDialyzer:

1. Disassemble the unit. Rinse the plastic pieces with distilled water. Keep the snap clamps separate from the plastic pieces.
2. Soak the plastic parts over night in a mild surfactant solution. Spectrum recommends a solution of 1 fluid ounce of Ultraclean (Part No. 105544) per gallon distilled water. Avoid scratching or abrading the plastic surfaces.
3. Flush the parts with plenty of distilled water.
4. Rinse the plastic parts with 20 % ETOH and dry them with a soft cloth.

Sterilization

The dialysis membrane may be radiation sterilized.

To radiation sterilize the membrane, expose it to a gamma ray source until it has received a total dose of 2.5 megarads. During the exposure the temperature should not rise by more than 10 °C and should always remain below 35 °C.

Never expose a Spectra/Por CE membrane to ethylene oxide or temperatures above 37 °C. The CE membranes are soluble in ethylene oxide; temperatures above 37 °C will alter the MWCO of a CE membrane.

Dialysis Speed

Since the membrane area of a Spectrum MicroDialyzer is independent of the size of the sample placed into the well, the time required to dialyze a sample will depend upon the sample volume.
To illustrate the correlation between sample size and dialysis speed, an experiment was performed. The results are shown in Figure 4. In this experiment, a 16 well 150 µl MicroDialyzer was used with a 15,000 MWCO membrane. Three sample sizes (25 µl, 50 µl and 100 µl) of a 0.125 M NaCl, 0.200 M Tris, pH 7.0 solution were dialyzed against pure water. At predetermined time intervals, the conductivities of the samples were measured to determine the amount of salt remaining.

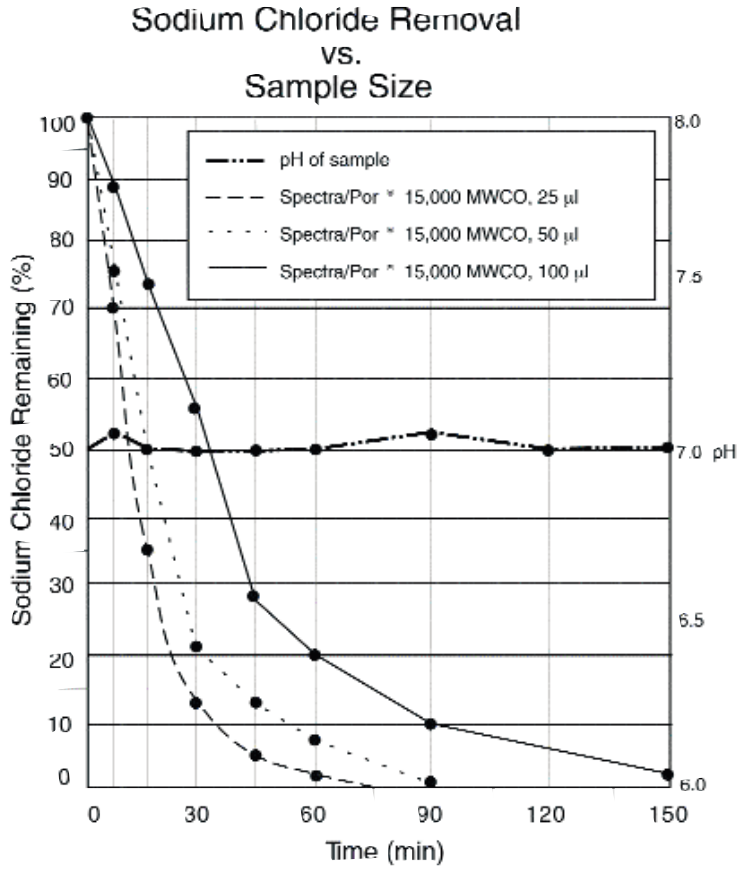

Figure 5 - Relationship between Sample Size and Dialysis Speed

Experimental System

Batch systems may be designed specifically to remove certain ionic species or macromolecules. In a batch operation the dialysate is continually monitored by a detection system so that the condition of the sample may be inferred. The dialysate will generally be pumped through the detector and recycled. The dialysis will be considered complete when the condition of the dialysate indicates that the sample has reached its desired condition.

Combining a MicroDialyzer, a MP-2 Pump, a Chart Recorder, and either

a Spectra/Chrom® pH/Ion Gradient Monitor or a Spectra/Chrom® UV Monitor yields an excellent system for the batch dialysis of small samples. The appropriate monitor provides information about the changes in the dialysate caused by changes in the sample, thus allowing the sample's condition to be inferred.

Specifications

	(# Samples/Volume)			
Well System	**10/150**	**10/500**	**16/150**	**96/150**
Membrane Surface Area/ Well (sq. mm)	24	65	24	24
Maximum Well Capacity (µl)	230	620	230	560
Minimum Well Capacity (µl)	24	65	24	24
Length (cm)	6.4	6.4	12.7	15.2
Width (cm)	6.4	6.4	6.4	13.3
Height (cm)	4.0	4.0	4.0	4.0
Chamber Vol. (ml)	30	32	34	112

Ordering Information

MicroDialyzers

Part No.	*Description*
132320	Spectra/Por® 10-Well MicroDialyzer, 150 µl capacity
132321	Spectra/Por® 10-Well MicroDialyzer, 500 µl capacity
132324	Spectra/Por® 16-Well MicroDialyzer, 150 µl capacity
132326	Spectra/Por® 96-Well MicroDialyzer, 150 µl capacity

Spectra/Por CE Membrane Frames for MicroDialyzers

MWCO	*For MicroDialyzers 10-Well*	*5, 12, 16-Well*	*96-Well*
100	132940	132955	132970
500	132941	132956	132971
1,000	132942	132957	132972
2,000	132943	132958	132973
3,500	132944	132959	132974
5,000	132945	132960	132975
8,000	132946	132961	132976
10,000	132947	132962	132977
15,000	132948	132963	132978
25,000	132949	132964	132979
50,000	132950	132965	132980
100,000	132951	132966	132981
300,000	132952	132967	132982

Cellulose Ester (CE) precut membranes comes individually packaged frames in 0.1% Sodium Azide in 10 frames/package.

MicroDialyzer Accessories

Part No.	Description
132882	5-Sample Well Plate, 500 µl
132327	10-Sample Well Plate, 150 µl
132328	10-Sample Well Plate, 500 µl
132883	12-Sample Well plate, 150 µl
132329	16-Sample Well Plate, 150 µl
132330	96-Sample Well Plate, 150 µl
132332	Sample O ring (for 150 µl), 20/pkg
132334	Sample O ring (for 500 µl), 20/pkg
132336	Replacement Kit for Spectra/Por® 150 µl/500 µl MicroDialyzer: 2 Luer Fittings, 2 Three-way Valves, 20 of 150 µl O rings, 10 of 500 µl O rings, and 2 Dialysis Chamber O-rings

Part No.	Description
185 850	SilentSwirl® Magnetic Stirrer, 100/115 V
146 821	MP-2 Pump, 115 V
146 822	MP-2 Pump, 230 V
146 811	Pump Head for 3/16 x 1/16 tubing, size: 14
123 744	Vinyl Tubing, size: 14, 3/16 x 1/16, 30 m
146 813	Pump Head for 1/4 x 1/8 tubing, size: 16
123 758	Vinyl Tubing, size: 16, 1/4 x 1/8, 30 m
146 823	Multiple Pump Head mounting hardware
123 454	UV Monitor 254/280, 115 V
123 457	UV Monitor 254/280, 230 V

Chemical Resistance

Classification:

G - Good chemical resistance.
F - Fair chemical resistance (changes in MWCO may occur)
N - Not recommended

As a general guide, Spectrum® recommends that Spectra/Por® CE membranes not be exposed to organic solvents stronger than a 5% solution in water. Some solvents cause no apparent harm to the CE membranes such as hydrocarbons and perhalohydrocarbons (i.e., carbon tetrachloride and Freons) and the lower alcohols. The latter, however, may change the MWCO.

The chemical resistance table given below is intended for use as a guide. The information is believed to be reliable, however, variables in temperature, concentrations, duration of exposure and other factors may affect the use of the product. You may wish to test the membrane under your own conditions.

	Cellulose Ester	Buna-N	Acrylic
Hydrocarbons			
Hexane, xylene	G	N	N
Toluene, benzene	G	N	N
Kerosene, gasoline	G	G	G
Tetralin, decalin	G	G	G
Halogenated hydrocarbons			
Methylene chloride	N	N	N
Chloroform	N	N	N
Carbon tetrachloride	F	F	N
Trichloroethylene	G	F	N
Perchloroethylene	G	F	N
Monochlorobenzene, Freon	G	F	N

	Cellulose Ester	Buna-N	Acrylic
Alcohols			
Methanol, 98%	N	G	N
Ethanol, 98%	N	F	N
Ethanol, 70%	N	F	F
Isopropanol, n-Propanol	N	F	F
Amyl alcohol, butanol	N	F	F
Benzyl alcohol	F	F	N
Propylene glycol	F	G	F
Ethylene glycol	G	G	F
Glycerol	G	G	F
Ketones			
Acetone, cyclohexanone	N	N	N
Methyl ethyl ketone	N	N	N
Isopropylacetone	N	N	N
Esters			
Ethyl acetate, methyl acetate	N	N	N
Amyl, propyl, butyl acetate	N	N	N
Propylene glycol acetate	N	N	N
Esters (cont.)			
2-Ethoxyethyl acetate	N	N	N
Methyl cellosolve acetate	N	N	N
Benzyl benzoate	G	N	N
Isopropyl myristate	G	N	N
Tricresyl phosphate	G	N	N
Oxides			
Ethyl ether	G	N	N
Dioxane, tetrahydrofuran	N	N	N
Dimethylsulfoxide	N	N	N
Solvents containing nitrogen			
Dimethyl formamide	N	F	N
Dimethylacetone	N	N	N
Triethanolamine	G	G	N
Aniline	N	N	N
Pyridine	N	N	N
Acids			
Hydrochloric acid, 30%	N	F	F
Hydrochloric acid, 25%	N	F	F
Nitric acid, 65%	N	N	N
Nitric acid, 25%	N	N	F
Sulfuric acid, 96%	N	N	N
Sulfuric acid, 25%	N	N	G
Phosphoric acid, 25%	G	N	F
Formic acid, 100%	N	N	N
Formic acid, 25%	N	N	F
Acetic acid,100%	N	F	N
Acetic acid, 25%	N	F	F
Trichloroacetic acid, 10%	G	F	N
Trichloroacetic acid, 25%	N	F	N
Perchloric acid, 25%	N	N	N
Hydrofluoric acid, 25%	G	F	N

	Cellulose Ester	Buna-N	Acrylic
Alkalies			
Ammonium hydroxide, 25%	N	N	G
Ammonium hydroxide, 1 N	N	N	G
Sodium hydroxide, 1 N	N	F	G
Potassium hydroxide, 1 N	N	F	G
Miscellaneous			
Aqueous phenol, 10%	N	N	
Solvent E 33 (Hoechst)	N		
Methyl cellosolve	N	F	N
Formaldehyde solution, 30%	F	F	N
Turpentine, castor oil	G	G	
Peanut oil	G	G	
Silicone oil, mineral oil	G	G	
Hydrogen peroxide, 30%	G	F	G

References

acey, Dr. R.I. 1978., Dept. of Physiology-Anatomy, University of California, Berkeley, *Membrane Disorders*, ed. by Thomas E. Andreoli, M.D.

Notice to 96-Well Dialyzer Users
Part No. 132326

To facilitate assembly of the dialysis unit and to improve seal effectiveness, the 96 silicone O-rings have been replaced with a one piece sheet gasket composed of 1/8" thick, closed cell BUNA-N. Three reusable gaskets are provided for use with the polystyrene framed membranes.

Gasket Installation Instructions:

1. Use instructions described in "Use of Dialyzer Equipped with Thumb Screw" section of the DIRR. Follow steps 1 through 3. Note that O-rings are no longer required.

2. At step 4, first position the BUNA-N Gasket on the alignment pins of the dialysis chamber plate. Position the framed membrane as described in step 4.

3. Complete assembly and operation of unit as described in steps 4 though 11.

4. BUNA-N Gaskets may be used repeatedly until they become permanently compressed and no longer seal effectively. Three gaskets are provided with each dialyzer unit. Each package of 10 polystyrene framed membranes includes additional gaskets.

How To Place an Order

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