SpectraPor® Regenerated Cellulose Dialysis Membrane

User Guide

Standard Grade 1 - 7





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Abbreviations

EtO Ethylene oxide

EDTA Ethylenediamineetetraacetic

FW Flat Width

MWCO Molecular weight cut-off

MW Molecular weight
PTFE Polytetrafluoroethylene
RC Regenerated Cellulose

1. Introduction

SpectraPor® Regenerated Cellulose (RC) Membrane is a versatile membrane available in a wide selection of sizes, molecular weight cut offs (MWCOs) and formats for laboratory scale dialysis and large volume process scale dialysis.

2. Membrane Composition and Specifications

SpectraPor RC Membranes 1- 7 are manufactured from natural cellulose reconstituted from cotton linters. SpectraPor RC Membranes carry no fixed charge and do not adsorb most solutes. RC membranes are used for general laboratory dialysis applications such as desalting, buffer exchange, or molecular separation. These flexible, transparent membranes feature good chemical compatibility as well as pH and high temperature tolerance.

2.1 Specifications

Table 1. Standard RC Dialysis Membrane Specifications

Membrane Type	Regenerated Cellulose
Physical Properties	Transparent, flexible
pH Range	2 - 12
Recommended Max. Temperature	60° C (operating)
Organic Solvent Tolerance:	Good

Table 2. SpectraPor Standard RC Dialysis Membranes

Membrane	мwсо	Characteristics	Configurations	Preparation
SpectraPor 1	6 - 8 kD	Standard permeability	Tubing, trial kits, discs and sheets	Dry with glycerin
SpectraPor 2	12 - 14 kD	Higher permeability or large FW	Tubing, trial kits, discs and sheets	Dry with glycerin
SpectraPor 3	3.5 kD	Standard permeability	Tubing, trial kits, discs and sheets	Dry with or without glycerin
SpectraPor 4	12 - 14 kD	Standard permeability	Tubing, trial kits, discs and sheets	Dry with glycerin
SpectraPor 5	12 - 14 kD	Reinforced with cellulosic fibers	Tubing and trial kits	Dry without glycerin
SpectraPor 6	1, 2, 3.5, 8, 10, 15, 25 and 50 kD	Pre-wetted for convenience	Tubing and trial kits	Wet in 0.05% sodium azide
SpectraPor 7	1, 2, 3.5, 8, 10, 15, 25 and 50 kD	Pre-treated for heavy metals and sulfides	Tubing and trial kits	Wet in 0.05% sodium azide

For information and purchase of any dialysis product visit the Repligen estore (store.repligen.com).

Table 3. Metal content in SpectraPor RC Dialysis Membranes 1 - 6

Element	Concentration
Cd	< 1 ppm
Cr	< 1 ppm
Cu	< 1 ppm
Fe	20 - 60 ppm
Mg	50 - 100 ppm
Ni	1 - 2 ppm
Pb	2 - 6 ppm
Zn	5 - 10 ppm

The following are approximate concentration of heavy metal and sulfur found in the SpectraPor RC Membranes 1 - 6.

2.2 Chemical Compatibility

SpectraPor RC Membranes have good chemical compatibility. Variables such as temperature, concentrations, durations of exposure and other factors may affect the membrane tolerance to a chemical. It is recommended to test the membrane under your application conditions. RC membranes are resistant to the following groups: halogenated hydrocarbons, alcohols, ketones, esters, oxides and solvents containing nitrogen. For a complete chart of chemical compatibility visit the dialysis section of repligen.com.

3. Membrane Selection

3.1 Membrane Permeability

Dialysis membranes are universally characterized by the MWCO, the performance rating of the membrane. The MWCO of the membrane is determined as the molecular weight of the smallest solute which is at least 90% retained during this test (the smallest solute for which the permeation is 10% or less).

The solute molecular weight (MW) has a direct impact on the rate of dialysis. The smaller the solute MW with respect to the MWCO, the more readily it passes through the membrane resulting in a faster rate of dialysis. The larger the solute MW, the longer it will take to pass through the membrane resulting in a slower rate of dialysis. As the solute size nears the MWCO, the rate will slow dramatically until the molecules become too large to pass through the membrane.

3.2 MWCO Selection

MWCO values are not absolute values and will change based on conditions and interaction between solute and sample. The effective size of many solute molecules will be affected by the pH and ionic strength of the solution in which they are dissolved. It may be necessary to test several MWCOs to determine the optimal membrane for a particular application. To maximize the rate of dialysis, select a membrane with the largest MWCO that will not cause excess loss of the desired molecular species.

MWCO selection is based on the MW of the larger solutes to be retained inside the membrane and the MW of the smaller solutes to be removed from the sample. For more tips on membrane selection visit the frequently asked questions section of repligen.com

3.3 Flat Width (FW) Selection

The selection of the tubing FW depends on the sample volume and the size of the dialysis reservoir. Since a dialysis tubing with a smaller FW and corresponding diameter will have a higher surface area to volume ratio, it may dialyze more quickly compared to a dialysis tubing with a larger FW and diameter due to the longer diffusion distances involved. For easy handling of the dialysis tubing, the suggested total length including closures and head space should be approximately 10 - 15 cm. The volume/length ratio (ml/cm) is provided on our website for each FW.

3.4 Configurations

Table 4. Rolled Tubing and Trial Kit Configurations

Description	SpectraPor 1 - 5	SpectraPor 6	SpectraPor 7
Rolled Tubing:	15 m or 30 m	10 m	5 m
Trial Kits with a Pair of Closures:	1 m or 5 m	1 m	1 m

- **Ready-to-use Dialysis Sacks*:** 60 cm tubing length sealed with a closure at the bottom and open with attached funnel at the top for easy sample filling. 10 sacks/pkg in 0.05% sodium azide solution (rinse before use)
- Membrane Discs and Flat Sheets*: 1 ply membrane cut to specific shapes
 - Precut membrane discs 50 per package
 - Flat sheet membranes 25 per package
 - Membrane supplied dry with glycerol as a humectant

4. Closure Selection

Select a closure width of 4 - 10 mm longer than the flat width of the membrane tubing. This will allow the closure to seal the tubing securely when the dialysis tubing is loaded with sample solution. Closures are available in two types: SpectraPor (polypropylene) closures and Universal (nylon) closures.

4.1 SpectraPor Closures (Polypropylene)

The following SpectraPor® Closures are only for use with RC membranes:

- Standard Closure: Aids in sample buoyancy when applied to the top end of the dialysis tubing
- Weighted Closure: Contains a PTFE coated stainless-steel weight to keep dialysis tubing in a vertical floating position when applied to bottom end
- Magnetic Weighted Closure: Contains a PTFE coated magnetic bar, eliminating the need for a magnetic stir bar when the magnetic weighted closure is applied at the bottom end of the dialysis tubing

4.2 Universal Closures (Nylon)

Universal Closures may be used for all types of membrane tubing.

Since nylon sinks in water, use of Universal Closures requires 10-20% volume of air headspace to ensure sample buoyancy. Universal closures are not autoclavable.

5. Membrane Preparation

5.1 Rinse Procedure

For most applications, the membrane can be soaked in deionized water at room temperature for 30 minutes to remove the preservative (glycerin or sodium azide). Then, rinse membrane thoroughly with running deionized water.

5.2 Optional: Membrane Sterilization

The common method of membrane sterilization includes exposure to ethylene oxide (EtO) gas, irradiation or autoclaving. Membranes may be autoclaved at 121° C for no more than 15 minutes (cycle should be kept as short as possible) immersed in distilled water. However, it is essential that steam autoclaved RC membranes should be recharacterized prior to use to test changes in membrane performance. Repligen does not recommend boiling or prolonged exposure to temperatures greater than 60° C. Repligen offers pre-sterilized customized membrane. Contact your local sales representative or go to repligen.com for more information.

^{*}Not available for all membrane types, FW or MWCOs.

5.3 Optional: Heavy Metal Removal

Refer to <u>Table 3</u> for approximate concentrations of trace levels heavy metal and sulfur in the SpectraPor RC Membranes 1-6. SpectraPor RC Membranes 1-6 can be treated to remove heavy metals. Rinse and soak membrane in a 0.1% EDTA solution for 1 hour. Then rinse and soak membrane with deionized water for 15 minutes.

6. Membrane Handling and Use

6.1 General Protocol

The following is a general protocol for typical dialysis. There are many variables that should be considered before starting dialysis; including solute concentrations, sample and dialysate volumes, solvents, chemical compatibility, temperature, etc. Therefore, some application-specific changes to the following dialysis procedure may be necessary.

- 1. Fill a SpectraPor Reservoir with a large volume of appropriate dialysate (buffer). The dialysate volume should be ≥ 100X the sample volume. (Example: dialyze 10 ml sample in 1 L dialysate.)
- 2. Place an appropriately sized magnetic stir bar in the reservoir and place reservoir on a magnetic stir plate. Turn on the stir plate and adjust stirring speed to form a small, gentle vortex.
- 3. Cut dialysis tubing into an appropriate length based on sample volume. Allow 10 20% extra tubing length for air head space to ensure sample buoyancy above the rotating stir bar. Prepare the tubing according to the directions for use.
- 4. Open the first closure and apply to the dialysis tubing 3 5 cm from the bottom.
- 5. Load the sample through the open end of the dialysis tubing. Apply the second closure at least 5 cm from the top end of the dialysis tubing while allowing enough head space for buoyancy.
- 6. Place the dialysis sample in the dialysis buffer ensuring that the sample remains buoyant and does not interfere with the buffer stirring. Adjust stirring speed as necessary.
- 7. Typically, dialysis continues overnight. The dialysate (buffer) may be changed several times during dialysis, making sure to allow 2 4 hours of dialysis after the last dialysate change. Samples with higher contaminant concentrations, may need to dialyze for a longer duration with more frequent changes of dialysate solution.

Avoid in-process handling and manual buffer changes using SpectraFlo™ Dynamic Dialysis Systems. Visit <u>repligen.com</u> for more information.

6.2 Sample Recovery

Hold the portion of the tubing that extends above the top closure, open the top closure and then carefully pipette or pour out the sample into a container.

7. Membrane Storage and Shelf Life

Since the useful life of dialysis membranes can be affected by storage conditions, it is important to store under optimal conditions.

Dry membrane (SpectraPor RC Membrane 1 - 5) should be stored in original packaging at ambient conditions protected from moisture and extreme temperatures, or in a polyethylene bag at 4 - 8 °C. Properly stored dry membrane has a shelf life of 5 years.

Wet membrane (SpectraPor RC Membrane 6 and 7) and dry membrane (SpectraPor RC Membrane 1 - 5) that has been wetted for storage should be stored at 4 - 8 °C in a solution of 0.05% sodium azide, 1% sodium benzoate or 1% formaldehyde. This preservative solution should be changed at least every 6 months. Ensure that the storage solution does not freeze since ice crystals can permanently damage the membrane. If solution freezes at any point, the membrane should be discarded and not used. Properly stored wet membrane has a shelf life of 3 years.

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