SpectraPor® Biotech Grade Dialysis Membranes

User Guide

Membrane types:

- Cellulose Ester (CE)
- Regenerated Cellulose (RC)

REPLIGEN
INSPIRING ADVANCES IN BIOPROCESSING

The information contained in this document is subject to change without notice.

Repligen Corporation makes no warranty of any kind with regards to this material, including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose.

Repligen Corporation shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

No part of this document may be photocopied, reproduced, or translated to another language without the prior written consent of Repligen Corporation.

Products are not intended for diagnostic or therapeutic use or for use in vivo with humans or animals.

For further information, please contact Repligen Corporation at www.repligen.com.

Customer Support
customerserviceUS@repligen.com
310-885-4600

Repligen Corporation
18617 South Broadwick Street
Rancho Dominguez, California 90220
www.repligen.com

Contents

List of tables

Abbreviations

CE	Cellulose Ester
FW	Flat Width
kD	Kilodalton
MWCO	Molecular weight cut-off
MW	Molecular weight
pH	Potential of Hydrogen
PTFE	Polytetrafluoroethylene
RC	Regenerated Cellulose

1. Introduction

SpectraPor® Biotech Membranes are a higher purity dialysis tubing free of trace metals and sulfur compounds. Biotech Cellulose Ester (CE) is available in a broad range of MWCO's that provide better molecular separations for more challenging and critical dialysis applications. Biotech Regenerated Cellulose (RC) offers the additional benefit of a better chemical compatibility, as well pH and temperature tolerance.

2. Membrane composition and specifications

2.1 Specifications

Table 1. Biotech membrane specifications

Specification	Biotech RC	Biotech CE
Membrane type	Regenerated Cellulose	Cellulose Ester
MWCO	3.5 - 5, 8 - 10, 20 and 50 kD	0.1 - 0.5, 0.5 - 1.0, 3.5 - 5, 8 - 10, 20, 50, 100, 300 and 1000 kD
Physical appearance	Clear	Clear to translucent (≤ 20kD) Translucent to opaque (≥ 50kD)
Chemical compatibility	Good	Fair
Packaging	Dry with Glycerin (humectant)	Wet with 0.05% Sodium Azide
Flat width	10 and 16 mm	10, 16, 24 and 31 mm
pH limits	2 - 12	2 - 9
Suggested temperature limit	60° C	37° C

2.2 Chemical compatibility

Biotech CE membranes have fair chemical resistance compared to RC membranes. Variability in chemical concentrations, temperature, and exposure time as well as other factors may affect membrane tolerance, durability, and performance. Biotech CE membranes are generally compatible with the following groups: common alcohols (low to mid concentration), many dilute acids and bases and some dilute organics.

Biotech RC membranes have good chemical resistance to the following groups: hydrocarbons, halogenated hydrocarbons, alcohol, ketones, esters, oxides, and solvents containing nitrogen. RC membranes are not recommended for use with Hydrochloric Acid > 25%, Nitric Acid > 25%, 96% Sulfuric Acid, 25% Perchloric Acid, 1N Potassium Hydroxide and 10% Aqueous Phenol.

It is incumbent upon the user to verify chemical compatibility prior to use with a membrane. For specific chemical compatibility information visit the dialysis section of repligen.com.

3. Membrane selection

3.1 Membrane permeability

The pore rating and performance of a dialysis membrane is universally characterized by the Molecular Weight Cut-Off (MWCO). The MWCO of the membrane is determined by the molecular size that is retained approximately 90% by the membrane (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 and overall separation efficiency. While smaller MW solutes, in respect to the MWCO, will pass through the membrane faster, larger MW solutes will pass through slower and may require more time.

MWCO performance is not an absolute value and may change based on conditions and interaction between solute and sample. The effective size of many solute molecules may be affected by the pH and ionic strength of the solution in which they are dissolved.

3.2 MWCO selection

The MWCO selection is based on both the MW of the larger solutes to be retained by the membrane, as well as the MW of the smaller solutes to be removed from the sample. To maximize both yield and purity of the product of interest, the MWCO should be 80 - 90% of the larger solute MW and at least 50 times larger than the smaller solutes to be eliminated.

It may be necessary to test several MWCO's to determine the optimal membrane for an application. Trial Kits are an excellent option for comparing different MWCO's. To achieve better yield, select a lower MWCO that may sacrifice a little purity. Extending the duration of dialysis while using a lower MWCO may improve the purity. To achieve better purity, select a higher MWCO that may sacrifice a little yield. Shortening the duration of dialysis while using a higher MWCO may improve the yield. For more tips on membrane selection visit the frequently asked questions section of repligen.com.

3.3 Flat width (FW) selection and tubing length

The selection of the dialysis tubing FW depends on the sample volume and the size of the dialysis reservoir. However, for a given sample volume, a narrower flat width and therefore a longer tubing length will have a larger surface area. This may exhibit quicker and more efficient dialysis compared to a shorter length of dialysis tubing with a larger FW. For traditional static dialysis, a FW with corresponding volume capacity (ml/cm) should be selected to achieve a suggested total tubing length (including closures and headspace) of 10 - 15 cm that accommodates the sample volume. The "volume/length" ratio (ml/cm) is also provided on our website for each dialysis tubing FW.

3.4 Packaging configurations

SpectraPor® Biotech Membranes are available either as longer length rolled tubing for periodic dialysis or shorter length dialysis trial kits, ideal for membrane and MWCO evaluation.

Biotech CE: Packaged prewetted in a preservative solution of 0.05% sodium azide:

- **Dialysis Trial Kit:** 1 m length with a pair of closures
- **Rolled Dialysis Tubing**: 10 m length

Biotech RC: Packaged dry with glycerin as a humectant to prevent brittleness:

- **Dialysis Trial Kit:** 0.5 m length with a pair of closures
- **Dialysis Rolled Tubing:** 5 m length

4. Tubing closure selection

To ensure a look-proof seal, select a tubing closure with a sealing width 4 - 10 mm longer than the flat width of the dialysis tubing. Closures are available in two types: SpectraPor® (Polypropylene) and Universal (Nylon).

4.1 SpectraPor® Closures (polypropylene)

The following SpectraPor® Closures are only for use with Standard and Biotech 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 of the dialysis tubing.
- **Magnetic Weighted Closure:** Contains a PTFE coated magnetic bar, eliminating the need for a magnetic stir bar when 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 but are required for use with Biotech CE. Since nylon sinks in water, use of Universal Closures requires 10 - 20% volume of air headspace to ensure sample buoyancy during dialysis. Universal closures are not autoclavable.

5. Membrane preparation

SpectraPor® Biotech Membranes only require a brief soak or rinse in DI water and/or dialysate buffer prior to use. Care must be taken prevent the wetted membranes from drying out. Wet membranes that have dried out will become brittle and may easily break during use.

1. Cut off an appropriate length of dialysis tubing (refer to Section 3.3).
2. Soak the dialysis tubing in water for 15 - 30 minutes at room temperature to remove Glycerin from dry Biotech RC or Sodium Azide from wet Biotech CE.
3. After soaking, rinse inside and outside the dialysis tubing thoroughly with deionized water. The membrane is ready for use.

6. Membrane sterilization

Biotech CE and RC dialysis tubing can be sterilized by either E-beam or Ethylene Oxide gas exposure. Biotech RC dialysis tubing can also be sterilized by autoclaving. However, it is incumbent upon the user to determine the appropriate treatment protocol and exposure dosage. It is recommended to recharacterize the membrane performance of autoclaved membranes.

As an option, Repligen provides pre-irradiated dialysis tubing as a customized membrane product for process dialysis applications. Contact customer service for more information.

7. Membrane handling and use

7.1 Traditional static dialysis

The following is a general protocol for static 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® Dialysis 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 the reservoir on a magnetic stir plate. Turn on the stir plate and adjust stirring speed to form a small, gentle vortex.
3. Cut the dialysis tubing into an appropriate length based on sample volume, allowing for 10 - 20% extra tubing length for air headspace to ensure sample buoyancy above the rotating stir bar (refer to Section 3.3.). Prepare the tubing according to Section 5.
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 headspace 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 needed.
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.

7.2 Dynamic Dialysis

SpectraFlo™ Dynamic Dialysis Systems provide a more efficient continuous buffer flow for larger dialysis sample volumes and tubing lengths longer than 15 cm. Dynamic dialysis is also a closed process that avoids in-process membrane handling and manual buffer changes. For more information on how to prepare and use a dialysis tubing with a SpectraFlo™ System, visit repligen.com/technologies/dialysis/spectraflo.

7.3 Sample recovery

1. Remove the dialysis tubing from the SpectraPor® Dialysis Reservoir or the SpectraFlo™ System.
2. Holding the portion of the dialysis tubing that extends above the top closure, remove the top closure, and then carefully pipette or pour out the sample into a container.

8. Membrane storage and shelf life

Dry membrane should be stored at ambient room temperature or at 4°C in a polyethylene bag. Avoid humidity. Properly stored, dry membrane has a recommended shelf life of 5 years.

Wet membrane 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 storage solution freezes, the membrane should be discarded and not used. Properly stored wet membrane has a recommended shelf life of 3 years.

9. Index