

Dialysis with CelluSep membranes

Directions for Use

For most applications, Cellu.Sep membranes can be used directly according [protocole 1](#). The membranes can be sterilized ([protocole 3](#)). If glycerol, sulfur compounds, or small amounts of heavy metals will interfere with subsequent steps, the membrane should be previously prepared as described below ([protocole 2](#)). For convenience with more sensitive applications, pre-treated, highly clean [Cellu.Sep H1](#) wet membranes are recommended.

Protocole 1: common use instructions of Cellu.Sep membranes

This protocol suits most classic applications with CelluSep membranes

- It is recommended to wear gloves
 - For Cellu.Sep T1.2.3.4
 - Cut the desired length of tubing from the roll
 - Soak and rinse briefly the inside with distilled water
 - Put a clamp (or make a knot) at one end of the tubing, secure it
- For Cellu.Sep H1 membranes (ready to use, sterile, and wet membranes),
 - Remove as many H1 membranes from the container as needed.
 - Rem: It is particularly important to store the unused membranes at 4-8°C with preservatives to avoid contaminations.
 - Carefully cut the individual pouch at one end and remove preservative solution (2% ethanol in distilled water)
 - Remove the membrane from its pouch and rinse with sterile distilled water.
 - Secure clamp to one end of the tubing. If a 50cm length is too long, cut down to appropriate length.
- Eventually check the integrity of the membrane by filling it with dialysis buffer and holding vertically. Pour out test solution.
- Introduce the sample to dialyse
- Put a clamp (or make a knot) at the top end, secure it.
- Note: Avoid trapping much air in the tube. For concentrated samples (salts), leave space enough in the tubing to allow for net flow of water into the sample and to prevent tubing from bursting
- Immerse the tubing in the dialysis bath (100-1000 times the sample volume) under constant stirring at room temperature (or +4°C if temperature-sensitive sample)
- Change the bath buffer as necessary. A general purpose protocol includes a first time dialysis period of 30min -1H, a second one of 1-2 hours, then a third overnight at 4°C
- Remove the tubing from bath, dry it briefly on an absorbant paper. Hold a tube end in the air, or directly above a container (tube, vial...). Open the clamp (or cut with clean scissors), allow the sample to flow out while pressing the tube between 2 fingers from closed end to open end. The interior of the tube might be rinsed with a small volume of buffer to increase recovery of small samples.

Note: The number of changes, the dialysis duration and the volumes of bath/sample might be optimized depending on operating conditions (temperature, agitation, sample/membrane type...) and desired dialysis efficiency/ application. Optimally, dialysis should reach the equilibrium state, but it usually requires a too long dialysis period. It is advantageous to shorten at least the first dialysis period(s). Viscous samples and rather hydrophobic molecules require longer dialysis time. For critical applications, one could monitor crucial compound absence/presence in the bath buffer/sample. For less exigent applications, sufficient dessalting may be obtained with only 2 (big bath) buffer changes. To estimate if dialysis volumes / changes may be acceptable, it is useful to calculate the dilution factor of the sample in the bathes, i.e. a 5ml sample is dialysed 3 times in 1liter, so the dilution factor is $(5/1000)^3 = 1.25 \cdot 10^{-7}$; if the sample contains initially 100mM Tris, the minimal final concentration is 12pM Tris (for 3 equilibrium dialysis steps).

Protocole 2: pre-treatment of Cellu.Sep membranes

- Wearing gloves, cut the desired length of tubing from the roll
- Soak for 15min in distilled water
- Heat to 80°C and incubate for 30minutes while stiring in 10mM sodium bicarbonate
- Incubate the tubing in a 10mM Na₂. EDTA solution for 30 minutes
- Repeat 3 times a 30min incubation in a fresh 10mM Na₂. EDTA solution
- Incubate for 30min at 80°C in distilled water under constant stirring
- Cool, and store in a refrigerator in a 0.5% sodium azide solution, or a 0.1% sodium benzoate solution, or alternatively in a 20-50% ethanol solution.
- The tubing should always remain submersed
- Before use, wash tubing inside and out with dialysis buffer. If necessary, tubing may be sterilized (see protocole 3)
- Follow protocole 1

Protocole 3: Sterilization of Cellu.Sep membranes

The common method of sterilization is exposure to ethylene oxide gaz. Alternative methods are either gamma irradiation, and steam autoclaving. Suggested preparation of membrane before sterilization is to soak it for 30min in distilled water.

Protocole 3a: Chemical sterilization with ethylene Oxide

Place the soaked membrane in an open polyethylene bag in a vacuum oven.

Evacuate and fill the oven with a gas mixture of 20% Ethylene Oxide + 80% CO2 by a total pressure of 1 atmosphere.

Treat the membrane for 5 hours at 40°C. Evacuate the sterilizing gas and admit 50% of humidity of air.

A slight reduction, approximately 10% permeability characteristic, has been reported with the use of this Ethylene Oxid method

Protocole 3b: Gamma-Irradiation sterilization

Seal the membrane in a polyethylene bag

Expose the bag to gamma ray source for a total dose of 2.5 Megarads. The temperature should not go beyond 10°C.

The permeability characteristic after the treatment is approximately 75%.

Protocole 3c: autoclaving sterilization

The membrane is treated with boiling water or steam autoclaving (121°C at 100kPa (41bar) for 10min in distilled water; The length of the cycling should be as short as possible). Autoclaved membrane should not be dried.

Uptima does not recommend this method, because temperature over 90°C will change the structure of the membrane. the permeation characteristics / performances should be recharacterized. Dry heating over a period of 48hours at 80°C drops the permeability to about 50%.

Technical Information

Compatibilities Technical Information

Chemical compatibility of CelluSep membrans with substances

Regenerated cellulose has excellent resistance against organic solvents. It suits too for aqueous solution and most salts, but care should be paid to the presence of acids and bases.

Symbols:

(Contact time : 24 hours at 20°C)

C= Compatible

L=Limited compatibility (swelling or shrinkage may occur)

N=Not compatible

Solvents		Bases	Miscellaneous
Acetonitrile	Isobutanol	Ammonium hydroxide 1N	Ammonium fluoride 20%
Benzene Benzyl alcohol	Isopropanol	Ammonium hydroxide 25%	Ammonium persulfate
n-Butanol	Isopropylacetate	Potassium hydroxide 32%	Ferric chloride 25%
n-Butyl acetate	Methanol 98%	Sodium hydroxide 32%	Formaldehyde 30%
Carbone tetrachloride	Methyl acetate	Sodium hydroxide 1N (NaOH)	Hydrogen peroxide 35%
CelloSolve	MethyleneC hloride		Sodium hypochloride 5M
Chloroform	MethylEthylKetone	Acids	
Cyclohexane	MethylisobutylKet one	Acetic acid 96%	
Cyclohexanone	Monochlorbenzene	Hydrochloric acid 37% (HCl)	
Diethylether	NitroBenzene	Hydrofluoric acid 25%	Amyl acetate
Diethylacetamide	n-Pentane	Hydrofluoric acid 50%	Amyl Alcohol
Diethylformamide (DMF)	Perchloroethylene	Nitric acid 65%	Freon
Dimetyl sulfoxide (DMSO)	Pyridine	Perchloric acid 25%	Glycerol
Dioxane	Tetrahydrofuran	Phosphoric acid 85%	Iodine Solutions
Ethanol 98%	Toluene	Sulfuric acid 25%	Urea
Ethyl acetate	Trichloethane	Sulfuric acid 98%	
EthyleneGlycol	Trichlo rethylene	Trichloroacetic acid 25% (TFA)	
Gasoline	Xylene		
Glycerol		Chloroacetic acid	
n-Heptane	Silicone oil	Hydrochloric acid 5% (HCl)	
n-Hexane	Oils mineral	Lactic acid	
	Ethylene Oxide	Hydrochloric acid 5% (HCl)	
	Ethers	Sulfuric acid 5%	
	Acetone	Chloroacetic acid	
Solvents		Acids	

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Scientific and Technical Information

Manufacturing information

Cellu•Sep® Dialysis tubings are regenerated cellulose membranes manufactured from select cotton linters. Cotton linters are 85-90% cellulose and contain fewer noncellulosic residuals than cellulose derived from wood fibers.

Rigorous chemical processes are used to obtain highest quality. After carefully selecting the cotton fiber diameter, length and blend, the pulp is refined under a proprietary process and extruded into tubular forms and flat sheets. Flux, wall thickness and porosity are monitored and controlled. The final product is a regenerated cellulose membrane with specific characteristics in strength, porosity and reliability.

Principle of dialysis

The dialysis process of a solute occurs by the movement of small molecules (solute) through a semi-permeable membrane governed essentially by the concentration differences on either sides, and the molecular weight of solutes. While big molecules are not allowed to cross the membrane, small solutes move randomly (diffuse according Newton rules), collide from time to time with the membrane until they go through pores. The net rate of transfer from one side to the other is affected by numerous variables:

- temperature
- viscosity of the solution
- mixing rate
- nature, ionic charge, size and shape of the solutes
- concentration differential
- membrane surface

Nominal Filter Rating

The most interesting and critical parameter corresponds to the molecular size below which molecules pass freely, and above which molecules are completely unable to pass (Molecular Weight Cut Off : MWCO). The pore size of a regenerated cellulose membrane have dispersed molecular cut-off size around a defined value, and the cut off depends on the solute nature. Thus MWCO are given as a range.

The 'Nominal Filter Rating' is a general and mean value where ca 90% of molecules with a lower molecular weight pass, and 90% are retained.

Membrane	MWCOs (Da)	Nominal Filter Rating (Da)
CelluSep T 1	4000-6000	3500
CelluSep T2	8000-10000	6000
CelluSep T1	12000-14000	12000

Performances

Cellu•Sep® Dialysis tubings permit rapid dialysis thanks to thin structure. The rate, extend and completion of dialysis can be estimated by using precipitation reaction, for example of chloride ions after silver nitrate addition.

Regenerated cellulose Cellu.Sep membranes has excellent resistance against most organic solvents, aqueous solutions, excepted in presence of acids and in a lower extend of bases or other compounds (see [compatibilities](#)). They have a very low non-specific adsorption, approximately 1µg/ml has been observed with bovine serum albumin.

Rem: CelluSep membranes are most susceptible to microbes and fungi, when wet. Such microbial growths will impair the dialysis properties and cause decreased yields and infection of the sample. Therefore, CelluSep membranes are offered with preservatives, and it is recommended to add preservatives for long term use, and storage (Bacterial growth will too be reduced at +4C). Sodium azide is a popular bacteriostatic for laboratory reagents, but it is considered highly toxic (LD50 = 45mg/kg orally in rats). Sodium benzoate (C7H5NaO2) is an interesting alternative in wet stored membranes because is less toxic (LD50=4.07 g/kg orally in rats; used at 1/1000 w/w in foods) and satisfactory efficient (and more efficient in acidic media).

Other Information

Reagent for R&D in vitro use only

Related products

[PBS \(powder pack, or tabs\)](#)

Contact uptima – Interchim for any question

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Contact your local distributor

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