

Introduction

MWCO filters are disposable ultrafiltration centrifugal devices for concentrating, desalting and buffer exchanging of biological samples such as proteins and nucleic acids. The filters contain a polyethersulfone (PES) membrane, which allows for processing of small volumes between 100 μ L and 750 μ L. The PES membrane is available in distinct molecular-weight cutoffs (MWCOs) of 1K, 3K, 5K, 10K, 30K, 50K, 100K, 300K. Typical protein recovery for proteins that are \geq two-fold greater than the MWCO is $> 90\%$. Sample concentration of 10- to 30-fold can be achieved in 10 minutes or less at 22 $^{\circ}$ C for MWCOs \geq 10K when centrifuging at 15,000 \times g. The filters compatible with most bench-top microcentrifuges.

MWCO Spin Filters - Micro - 800ul Capacity	
Rec. Tubes	With 2.0mL Receiver Tubes
Sterility: NON-STERILE	
Qty/Pkg: 100	
Membrane:	CAT. NO.
PES 1,000	8690-01
PES 3,000	8690-03
PES 5,000	8690-05
PES 10,000	8690-10
PES 30,000	8690-30
PES 50,000	8690-50
PES 100,000	8690-100
PES 300,000	8690-300
PVDF 30,000	7833-30
PVDF 100,000	7833-100
PVDF 250,000	7833-250

Important Product Information

- We recommend soaking the dry membranes in deionized water before use. There is no specific volume of water required, as long as the membrane is completely submerged. This duration will allow the membrane to re-wet and to allow the preservative to wash out.
- The filters contain a PES membrane rated for retaining molecules with a molecular weight \geq two-fold greater than the MWCO. Reduced recovery may occur with molecules that are $<$ two-fold greater than the MWCO. Recovery varies depending on the specific protein and starting concentration. Note: The PES membrane is effective for retaining molecules with molecular weights two-fold greater than the membrane's MWCO; therefore, for best results with IgG (MW~150K) samples, use the 3K, 10K or 30K MWCO concentrators.
- The filters can be used effectively at a maximum centrifugal force of 15,000 \times g.
- When centrifuging assembled concentrator, always place the device in the rotor in the same orientation.

- **Centrifugal force, temperature and sample volume, concentration and viscosity affect filtration rate. Optimize centrifugal time for each application.** A 0.25mg/mL protein sample will typically decrease in volume by \sim 12-fold in 5 minutes for the 10K and 30K MWCOs.
- The dead-stop volume for a 0.5mL sample is approximately 15 μ L. Note: The dead stop volume was determined for each MWCO using 0.2mg/mL protein with a MW two-fold higher than the cutoff. Note: Precipitation might occur at high concentration factors for some proteins. The maximum concentration factor is dependent on the specific protein, starting concentration and buffer system. Unless the stability of the protein has been determined, avoid concentrating to dead-stop.
- Do not allow the membrane to dry out following pre-rinsing. If the device is not used immediately, store it at 4 $^{\circ}$ C with buffer or water covering the membrane surface.
- Do not autoclave. High temperatures will significantly increase the membrane MWCO. To sterilize, use a 70% ethanol solution.
- The membrane is compatible with buffers at pH 1 to 9.
- The membrane is compatible with desalting and buffer exchange. The salt content can be reduced by $\geq 98\%$ with one exchange with water using the filters.

INSTRUCTIONS

Storage: Upon receipt store at room temperature. Product shipped at ambient temperature.

Procedure for Sample Concentration

- Centrifuge with a fixed-angle rotor for 2.0mL conical tubes, rated for up to 15,000 × g.
- Pipette for retentate (final sample) recovery (10-200µL tip).

Pre-rinsing

1. Add 500µL of buffer solution or purified water to the sample chamber.
2. Cap and insert sample chamber into a collection tube. Place filter assembly into rotor with a proper counterbalance.
3. Centrifuge at 15,000 × g.
4. Decant filtrate and retentate to remove buffer or water.
5. Replace concentrator into collection tube and proceed directly to Section C.
Note: Do not allow membrane to dry as performance may be affected.

C. Sample Processing

1. Place sample into the sample chamber.
2. Cap and insert sample chamber into collection tube. Place assembly into rotor with proper counterbalance.
3. Centrifuge at 15,000 × g until desired concentration factor is achieved. The following centrifugation times are recommended for concentrating protein solutions ≥ 0.1mg/mL approximately 10- to 30-fold.
Note: Reduce centrifugation time by half for 100µL sample volumes.
4. Use a 10µL or 200µL pipette tip to gently aspirate retentate from the sample chamber.

Procedure for Desalting/Buffer Exchange

- Centrifuge with a fixed-angle rotor for 2.0mL conical tubes, rated for up to 15,000 × g.
- Pipette for retentate recovery (10-200µL tip).
- Exchange Buffer.

Sample Processing

1. Place sample into the sample chamber. Sample volume range is 100µL to 750µL.
2. Cap and insert sample filter into collection tube. Place assembly into rotor with proper counterbalance.
3. Centrifuge at 15,000 × g until desired concentration factor is achieved. The following centrifugation times are recommended for concentrating protein solutions ≥ 0.1mg/mL approximately 10- to 30-fold in filters starting with a sample volume of 500µL.
Note: Reduce centrifugation time by half for 100µL sample volumes.
4. Dilute sample to original volume with Exchange Buffer.
5. Repeat Steps 3 and 4 until desired solute removal has been achieved.

Table 1. Sample Centrifugation Times

Concentrator MWCO	Sample Volume (µL)	Centrifugation Time (min)	Sample Volume (µL)	Centrifugation Time (min)
3K	500	30	100	15
10K	500	5	100	2.5
30K	500	5	100	2.5
100K	500	10	100	5

Table 2. Chemical Compatibility

<u>Acids and Bases</u>	<u>Rating</u>	<u>Organics</u>	<u>Rating</u>	<u>Miscellaneous</u>	<u>Rating</u>
Acetic acid (25%)	A	Acetone	NR	Ammonium sulfate (saturated)	A
Formic acid (5%)	A	Acetonitrile	NR	Glycerine (70%)	A
Hydrochloric acid (1M)	A	Benzene (100%)	NR	Guanidine HCl (6M)	A
Lactic acid (5%)	A	Chloroform (1%)	NR	Imidazole (300mM)	A
Nitric acid (10%)	A	Dimethyl sulfoxide(5%)	A	Phosphate buffer (1.0M)	A
Sodium hydroxide (2.5M)	NR	Ethanol (70%)	A	Polyethylene glycol (10%)	A
Sulfamic acid (5%)	A	Ethyl Acetate (100%)	NR	Sodium carbonate (20%)	A
Trifluoroacetic acid (10%)	A	Formaldehyde (30%)	A	Sodium deoxycholate (5%)	A
		Hydrocarbons (aromatic)	NR	Sodium dodecylsulfate (0.1M)	A
		Hydrocarbons (chlorinated)	NR	Sodium hypochlorite (200ppm)	A
		Isopropanol (70%)	A	Sodium nitrate (1%)	A
		Mercaptoethanol (1.0M)	NR	Tween [®] -20 (0.1%)	A
		Pyridine (100%)	NR	Triton [®] X-100 (0.1%)	A
		Tetrahydrofuran (5%)	NR	Urea (8M)	A
		Toluene (1%)	NR		

A = Acceptable **NR** = Not Recommended

*Concentrations listed are provided as guidelines and do not necessarily represent maximum tolerances. Some compatible chemicals might modify the apparent molecular weight of molecules in the sample and/or the molecular-weight cutoff rating of the membrane.

Troubleshooting

Problem	Possible Cause	Solution
Protein precipitation	Concentration was too high	Reduce concentration factor
		Try a different buffer system to increase protein solubility
Low protein recovery	Protein MW < two-fold higher than MWCO	Select a new concentrator with a MWCO at least two-fold lower than the protein MW
	Membrane damaged, protein in filtrate	Use a new concentrator and do not touch membrane with pipette tip
	<i>Note: A damaged membrane may exhibit a slightly higher than expected flux rate.</i>	Do not exceed recommended centrifugal force

