User Guide

Microcon® Biomax® PES Centrifugal Filter Device

For research use only. Not for use in diagnostic procedures.

Introduction

Microcon® Biomax® Polyethersulfone (PES) centrifugal filters provide efficient concentration, desalting, or buffer exchange of aqueous biological samples ranging in volume from 10–500 µL. A built-in deadstop prevents spinning to dryness and potential sample loss. Best performance is achieved using a centrifuge with a fixed-angle rotor.

Microcon® Biomax® PES devices are provided non-sterile.

Intended Use

Microcon® Biomax® PES centrifugal filter devices are for research use only. They are not for use in diagnostic procedures.

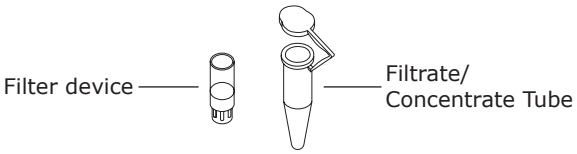
Application Guidelines

Use the following table to choose the correct Microcon® device for your application. Factors that should be considered are: Sample composition (i.e. buffers, salts, detergents); starting concentration; molecular complexity (other proteins in solution); Non Specific Binding, NSB: electrastatic/hydrophobic properies of the sample; Membrane Type using (Biomax® PES); and Membrane configuration (horizontal).

Applications by MWCO (K)

Reservoir Color	Yellow	Purple	Pink	Grey	Lime	Amber
Application	**5K**	**10K**	**30K**	**50K**	**100K**	**300K**
UF Concentration/ separation of biologicals (including Plants)	✓	✓	✓	✓	✓	✓
Protein separation (i.e. Transgenic plant-based expression systems for the manufacture of biopharmaceutical proteins)	✓	✓	✓	✓	✓	✓
Plant Polyphenol, Anthocyans & Flavinoids	✓	✓	✓	✓	✓	
Plant Membrane Vesicles				✓	✓	
Lysosomes (50 - 70 nm)					✓	✓
Protoplast (30 - 50 nm)					✓	✓
Chromatin isolation (10 - 30 nm)			✓	✓		
Buffer Exchange	✓	✓	✓	✓	✓	✓
Desalting	✓	✓	✓	✓	✓	✓
Enyme removal (ex. Cellulase [80 kDa], Esterase [168 kDa])	✓	✓	✓	✓		
Endotoxin removal (~10-20 kDa with larger aggregates)		✓	✓	✓		

Materials Supplied



Two tubes with attached sealing caps are included with each centrifugal filter device. During operation, one tube is used to collect filtrate, the other to recover concentrate.

Equipment Required

Any centrifuge that can properly accommodate 1.5 mL microcentrifuge tubes is acceptable, although fixed-angle rotors are preferred. A variable speed centrifuge is required for Microcon® Biomax® PES devices.

Suitability

Preliminary recovery and retention studies are suggested to ensure suitability for intended use. See the How to Quantify Recoveries section.

Device Storage

Store at room temperature.

Limitations

- Microcon® components are not autoclavable.
- Do not operate above the following limits, as excessive g-force may result in leakage or damage to the device:

Microcon® Biomax® PES Device	G-force
5K - 100K	14,000 x g
300K	5,000 × g

NOTE: G-force is not the same as RPM. Calculate g-force (relative centrifugal force or RCF) using this formula:

$RCF = 1.118 \times 10^{-6} \times radius \times (RPM)^2$

radius = distance in millimeters from the center of rotation to base of the filtrate tube

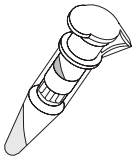
Rinsing Devices Before Use Not Needed

The ultrafiltration membranes in Microcon® Biomax® PES devices do not contain any glycerine so rinsing devices before use is not needed. If rinsing is still required, then rinse by adding 0.5 mL to the device with buffer or distilled water and spinning through before use.

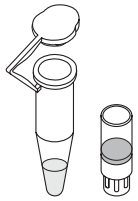
NOTE: Do not allow the membrane in Microcon® filter devices to dry out once wet. If you are not using the device immediately after rinsing, leave fluid on the membrane until the device is used.

How to Use the Microcon® Biomax® PES Filter

1. Insert Microcon® Biomax® PES device into tube.
2. Pipette solution into device (0.5 mL maximum volume), taking care not to touch the membrane with the pipette tip. Seal with attached cap.
3. Place assembly in a compatible centrifuge (described in the Equipment Required section) and counterbalance with a similar device. NOTE: When placing the assembled device into the centrifuge rotor, align the cap strap toward the center of the rotor.
4. Spin at 14,000 x g for Microcon® 5K to 100K or at 5,000 x g for 300K devices Refer to Table 1 for typical spin times.
5. Remove assembly from centrifuge. Separate tube from filter device.
6. Place a new tube over the top of the device. Invert the assembly and centrifuge for 3 minutes at 1,000 × g (or pulse briefly) to transfer concentrate to tube.
7. Remove from centrifuge. Separate tube from filter device. Close sealing cap to store sample for later use.

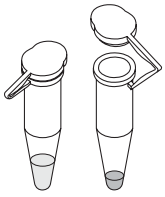


Assembled device during concentration spin



Individual components separated after spinning

Concentrate transfer during recovery spin



Filtrate and concentrate in sealable storage tubes

Spinning to Dryness

Extended centrifugation (2–3 times longer than guidelines) can lead to dryness. If this should occur, add at least 10 µL of buffer to the filter device, vortex for 10–30 seconds, then proceed with recovery.

Desalting / Diafiltration

Desalting, buffer exchange, or diafiltration are important methods for removing salts or solvents in solutions containing biomolecules. The removal of salts or the exchange of buffers can be accomplished in the Microcon® Biomax® PES device by concentrating the sample, discarding the filtrate, then reconstituting the concentrate to the original sample volume with any desired solvent. The process of "washing out" can be repeated until the concentration of the contaminating micro solute has been sufficiently reduced. Typically two spins, each concentrating the sample 20-fold, will provide 95% exchange of buffers or removal of low-molecular-weight contaminants.

Performance

Microcon® Biomax® PES centrifugal filter devices have been tested for flow rate, retention, and recovery with several well-known materials. Tables 1, 2 and 3 can be used to estimate device performance. Actual performance, however, depends upon the nature of the specific sample under study.

Flow Rate

Factors affecting flow rate include sample type and concentration, starting volume, relative centrifugal force, angle of centrifuge rotor, membrane type, and temperature.

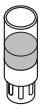
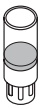
Table 1.
Typical Spin Times and Concentration Factors to ~10x*

Application	Device (kDa)	G-force (x g)	Typical Spin Time (minutes)
Protein (Lysozyme 14 kDa)	5	14,000	10
Protein (BSA 67 kDa)	10	14,000	5
Protein (BSA 67 kDa)	30	14,000	5
Protein (IgG 156 kDa)	50	14,000	10
Protein (IgG 156 kDa)	100	14,000	10
Dextran (2,000 kDa)	300	5,000	10

* These guidelines are for starting volumes of 500 µL. For starting volumes less than 500 µL, spin times will be shorter.

Spin Time Optimization

The following protocol uses weight to estimate concentrate volume, since for dilute solutions, 1 g = 1 mL. By weighing the device after multiple 2-minute spins, the spin time to achieve a desired final volume or concentration factor can be estimated. For a more comprehensive protocol on estimating performance, refer to the Direct Weighing Protocol. Optimization of the wash spin times may also be done as described below.

Protocol for Optimizing Spin Time		Example
1. Pre-weigh an empty Microcon® Biomax® PES filter device (without tube).		Weight of empty device = .609 g
2. Add sample and reweigh filled device, or if pipetting, record dispensed volume. Subtract the weight of the empty device (step 1) from the filled device to obtain the starting volume. Assemble device as indicated, and spin at the appropriate g-force for 2 minutes.		Weight of filled device = 1.109 g Starting volume = 1.109 g - 0.609 g 0.500 g (or mL)
3. Remove the filter device from the filtrate tube and weigh. Subtract the weight of the empty device (step 1) from the after-spin weight to obtain the concentrate volume.		Weight of device after spin = 0.859 g Concentrate vol. after 1st spin = 0.859 g - 0.609 g 0.250 g (or mL)
4. Reassemble device in filtrate tube and perform another 2-minute spin. Remove device from filtrate tube and reweigh. Subtract the weight of the empty device (step 1) from the after-spin weight to obtain the concentrate volume.		Weight of device after spin = 0.709 g Concentrate vol. after 2nd spin = 0.709 g - 0.609 g 0.100 g (or mL)
5. Continue with the 2-minute spins and device weighing until the desired concentrate volume or concentration factor (starting volume divided by final concentrate volume) is achieved. Total the 2-minute spin times to estimate the spin time required to achieve the desired concentrate volume or concentration factor.		Concentration factor 0.500 mL / 0.100 mL = 5X Total spin time 2 + 2 = 4 min.

Retention and Recovery

The Biomax® PES membranes in Microcon® centrifugal filter devices are characterized by a molecular weight cutoff (MWCO). MWCO is the ability of the membrane to retain molecules above a specified molecular weight. Solutes with molecular weights close to the MWCO may be only partially retained. Membrane retention depends on the solute's molecular size and shape. For most applications, molecular weight is a convenient parameter to use in assessing retention characteristics. We recommend using a membrane with a MWCO at least two to three times smaller than the molecular weight of the isolate or protein solute that one intends to concentrate.

Table 2. Typical Protein Recovery Microcon® Biomax® PES 5K - 300K Devices

Solute / Concentration	Molecular Weight	Typical % Recovery from Concentrate					
		5K	10K	30K	50K	100K	300K
Blue Dextran (1mg/mL)	2,000 K						97%
Bovine IgG Fraction II (1 mg/mL)	156 K				95%	95%	
Bovine serum albumin (1 mg/mL)	67 K			95%			
Ovalbumin (1 mg/mL)	45 K		93%				
α-Chymotrypsinogen (1 mg/mL)	25 K	94%	64%				
Lysozyme (1 mg/mL)	14.4 K	90%					
Cytochrome c (0.25 mg/mL)	12.4 K	73%					

Maximizing Sample Recovery

Low sample recovery from the concentrate may be due to adsorptive losses, over-concentration, or passage of sample through the membrane.

- Adsorptive losses depend on sample concentration, its hydrophobic nature, temperature and time of contact with filter device surfaces, sample composition, and pH. To minimize losses, remove concentrated samples immediately after centrifugal spin.
- If starting sample concentration is high, monitor the centrifugation process in order to avoid over-concentration of the sample. Over-concentration can lead to precipitation and potential sample loss.
- If the sample appears to be passing through the membrane, choose a lower MWCO Microcon® Biomax® PES device.
- For Microcon® Biomax® PES devices in concentration and desalting applications, concentration factors of 100x can be achieved.

How to Quantify Recoveries

Calculate total recovery, percent concentrate recovery, and percent filtrate recovery using the protocol below. This protocol provides a close approximation of recoveries for solutions having concentrations up to roughly 20 mg/mL.

NOTE: Appropriate assay techniques include absorption spectrophotometry, radioimmunoassay, refractive index, and conductivity.

Direct Weighing Protocol

The density of most dilute proteins is nearly equal to the density of water (i.e., 1 g/mL). Using this property, the concentrate and filtrate volumes can be quantified by weighing them and converting the units from grams to milliliters. This technique is valid only for solutions with concentrations of approximately 20 mg/mL or less.

1. Separately weigh the empty filter device, filtrate collection tube, and concentrate collection tube before use.
2. Fill filter device with solution and reweigh.
3. Assemble device in filtrate collection tube and centrifuge per instructions.
4. Collect the concentrate by inverted spin into the pre-weighed concentrate collection tube.
5. Remove the device from the concentrate collection tube and weigh the filtrate and concentrate collection tubes.
6. Subtract weight of empty device/tubes to calculate weights of starting material, filtrate, and concentrate.
7. Assay the starting material, filtrate, and concentrate to determine solute concentration.
8. Calculate recoveries using the weight/volume data and the measured concentrations as follows:

$$\%\ \text{concentrate recovery} = 100 \times \frac{W_c \times C_c}{W_o \times C_o}$$

$$\%\ \text{filtrate recovery} = 100 \times \frac{W_f \times C_f}{W_o \times C_o}$$

% total recovery = % concentrate recovery + % filtrate recovery

W_c = total weight of concentrate before assay

C_c = concentrate concentration

W_o = weight of original starting material

C_o = original starting material concentration

W_f = weight of filtrate

C_f = filtrate concentration

Specifications

Maximum initial sample volume	0.5 mL (500 µL)
Typical final concentrate volume	5–50 µL
Maximum relative centrifugal force	
Microcon® Biomax® PES 5K - 100K devices	14,000 × g
Microcon® Biomax® PES 300K devices	5,000 x g
Active membrane area	0.32 cm^2
Hold-up volume	≤ 10 µL
Dimensions	
Diameter	12.3 mm (0.5 in.)
Length (filter device and tube in concentration mode)	45.0 mm (1.8 in.)
Length (filter device and tube in recovery mode)	48.2 mm (1.9 in.)
Materials of Construction	
Membrane	Biomax® Polyethersulfone (PES)
Device top	Polycarbonate
Membrane support base	Acetal
Filtrate/concentrate tube	Polypropylene
O-ring	Medical-grade silicone rubber

Disposal

Microcon® Biomax® PES devices exposed to chemicals or biologics should be safely disposed of in accordance with local regulations.

As part of our commitment to increased environmental sustainability, this product is packaged in recyclable materials. Please dispose of responsibly.

Chemical Compatibility

The solutions listed in the table below have been evaluated for chemical compatibility in Microcon® devices containing Biomax® PES membranes. Contact with some organic chemicals may cause leaching from component parts. If leaching is suspected, run solvent blanks before performing assays.

Caution Please note the following:

1. These recommendations assume pure solutions at room temperature and pressure without applied stresses. Time of exposure is not considered and some should be limited or lower concentrations used. These are critical assumptions as polymer properties are strongly affected by environmental conditions, time, the presence of external stress and the presence of additives. It is not safe to assume that property changes are linearly related to changing temperature. A 10 °C increase in temperature, for example, may place the test conditions closer to the glass transition of the polymer, thus allowing greater penetration of solvent molecules. This has a plasticizing effect, further lowering the glass transition and resulting in a modulus drop of up to three orders of magnitude.
2. These recommendations assume that each polymer category has a uniform chemistry, molecular weight distribution and thermomechanical history. This assumption will never be true and, in some cases, variation has a distinct influence on compatibility. Crystalline morphology and degree of crystallinity influences compatibility of semi-crystalline polymers and can vary significantly. Such specific information concerning polymers evaluated does not accompany published compatibility tables.
3. The definition of solvent compatibility for our products differs from that used in determining the ratings given in published compatibility tables. Such tables are generally concerned with chemical attack and significant losses in strength and/or dimensional changes. A top designation, for example, might be designated for solvent-polymer combinations with <10% swelling, which is high. Other compatibility tables may make recommendations based upon dimensional change as a function of time. This is difficult to relate to a membrane that may respond almost immediately to immersion in solvent. In addition, solvent-membrane compatibility requires additional consideration of filtration-specific factors. None of these published compatibility guides, for example, monitors the solvent's ability to wet a membrane or increase extractables.
4. This table does not consider solvent safety issues.

Table 3. Chemical Compatibility

The following descriptions are abbreviated. Please see page 5 for complete information.

R = Recommended GR = Generally Recommended
NR = Not Recommended TST = Testing Recommended

	Chemical	Microcon® Biomax® PES
RECOMMENDED	Acetic Acid, glacial, (≤ 25%)	R
	Ammonium Hydroxide (5%)	R
	Ammonium sulfate (saturated) salt, aqueous solution	R
	Dimethylformamide (≤ 10%, limited)	GR
	Formaldehyde (≤ 30%), aldehyde	R
	Formic Acid (5%)	R
	Glycerine (70%)	R
	Guanidine Hydrochloride, 6M salt, aqueuos solution	R
	Hycrochloric Acid, 1 N (HCl) acid, inorganic	GR
	Hydrogen Peroxide, 3% peroxide	R
	Imidazole (300 mM)	R
	Latic Acid, 50% acid, organic/alcohol	R
	Phosphate Buffer (1M, pH 8.2)	R
	Phosphoric Acid (5%)	R
	Polyethylene Glycol (10%)	R
	Sodium Carbonate (aqueous solution) salt, aqueous solution	R
	Sodium Deoxycholate (5%)	R
	Sodium Dodecyl Sulfate surfactant/detergent	R
	Nitric Acid (10%)	R
	Sulfamic Acid (3%)	R
	Sulfuric Acid (3%)	R
	Terg-A-Zyme® (1%), detergent	R
	Trifluoroacetic Acid (10%)	R
	Tris® Buffer (1 M, pH 8.2)	R
	Triton® X-100 (5 mM)	R
	Water (Brine) salt, aqueous solution	R
	Mercaptoethanol (0.1 M)	R

	Chemical	Microcon® Biomax® PES
TEST	n-Butanol, Butyl alcohol (70%)	GR
	Hycrochloric Acid, 1 N (HCl) acid, inorganic	GR
	Dimethyl Sulfoxide (DMSO), (≤ 10% limited housing/100%)	GR/NR
	Dimethylformamide, amide (≤ 10% limited housing/100%)	GR/NR
	Isopropanol Alcohol (10%/70%)	R/GR
	Ethyl Alcohol (10%/70%)	R/TST
	Methyl Alcohol, Methanol (10%/60%)	R/TST
	Sodium Hydroxide (≤ 1N/> 1N)	GR/TST
	Ultrasil 11® detergent (0.5%)	TST
NOT COMPATIBLE	Acetone	NR
	Acetonitrile (ACN), nitrate	NR
	Benzene HC, aromatic	NR
	Chloroform HC, halogenated	NR
	Ethyl Acetate, ester	NR
	Hydrocarbons, aromatic	NR
	Hydrocarbons, chlorinated	NR
	Methyl Ethyl Ketone (MEK)	NR
	Pyridine, amine	NR
	Sodium Hydroxide, concentrated caustic	NR
	Tetrahydrofuran (THF), ether	NR
	Toluene HC, aromatic	NR
	Urea, 8 M salt, aqueous solution	NR

Troubleshooting / Optimization

Protein recovery in retained fraction is low

Possible Cause	Solution
Protein expression was insufficient.	Optimize growth/induction conditions.
Protein was insoluble (inclusion bodies).	Following lysate clearance, check the pellet and supernatant for protein. Perform cell lysis under denaturing conditions.
Protein formed aggregates.	Add solubilizing agents as detergents, or increase salt concentration of lysis and binding buffers.
Cell lysate was too viscous.	Dilute lysate in binding buffer. Add Benzonase® nuclease to lysis buffer to remove free RNA/DNA.
Sample bound non-specifically to the device.	Check chemical compatibility of buffers used.
Protein precipitated due to over-concentration.	Reduce centrifugation time during the concentration step.
Protein was lost during sample concentration.	Check the filtrate in the collection tube for protein. Verify the protein's expected molecular weight to confirm that the appropriate MWCO Microcon® Biomax® PES device was used.

Protein purity is poor

Possible Cause	Solution
Sample was degraded due to suboptimal culture conditions.	Optimize growth/induction conditions.
Sample was degraded due to suboptimal lysis conditions.	Optimize lysis parameters. Include protease inhibitors in lysis buffer.
Cell lysate was too concentrated.	Dilute lysate in buffer.
Cell lysate was too viscous.	Dilute lysate in binding buffer. Include Benzonase® nuclease in lysis buffer to remove free RNA/DNA.

Concentration of the contaminating microsolute is too high

Possible Cause	Solution
No wash steps were performed, or washing was insufficient.	Incorporate wash step(s) into sample processing. Increase volume of wash buffer. Supplement the wash buffer with detergents.

Sample is not flowing (filtering) through the device

Possible Cause	Solution
Sample was cold.	Non-heat sensitive samples: Bring sample to room temperature prior to spinning in centrifuge. Heat sensitive samples: Increase centrifugation time for cold centrifuge conditions.
Sample was too viscous.	Dilute sample in a compatible buffer.

Product Ordering Information

This section lists the catalogue numbers for Microcon® Centrifugal Filter Devices and related products. See the Technical Assistance section for contact information. You can purchase these products on-line at SigmaAldrich.com.

Description	Qty/ pk	Catalogue Number
Microcon® Biomax® PES, 5K Device	25	MPE005025
Microcon® Biomax® PES, 10K Device	25	MPE010025
Microcon® Biomax® PES, 30K Device	25	MPE030025
Microcon® Biomax® PES, 50K Device	25	MPE050025
Microcon® Biomax® PES, 100K Device	25	MPE100025
Microcon® Biomax® PES, 300K Device	25	MPE300025
Microcon® Biomax® PES Variety Pack includes: (4) 5K, (4) 10K, (4) 30K, (4) 50K, (4) 100K, and (4) 300K	24	MPEVAR024
Benzonase® Nuclease Purity >99%		70664
Benzonase® Nuclease HC, Purity >99%		71206
Benzonase® Nuclease Purity >99%		70746
Benzonase® Nuclease HC, Purity >90%		71205

Notice

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Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Assistance

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.