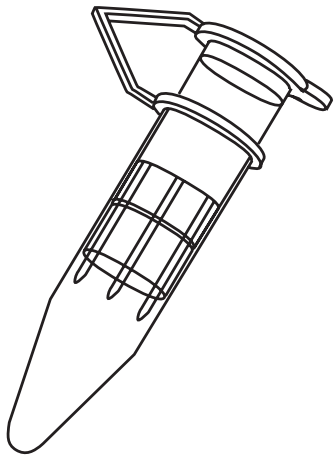
MICROCON®

Centrifugal Filter Devices

User Guide



**For research use only,
not for use in diagnostic procedures**

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Contents

Introduction

Microcon centrifugal filter devices simply and efficiently concentrate and desalt macromolecular solutions of 10–500 µL using any centrifuge that can accept 1.5 mL tubes. For best performance, use a centrifuge with a fixed angle rotor. If using Microcon devices with Ultracel® YM-100 membrane to process DNA or RNA, a variable-speed centrifuge is required.

With a medical-grade silicone rubber O-ring sealing the membrane, Microcon centrifugal filter devices can be spun as many as three times. See the "Chemical Compatibility" section for solvent resistance.

Introduction, continued

To prevent mix-ups in the lab, each Microcon centrifugal filter device's sample reservoir is color coded. The Microcon product line currently includes devices with the following Ultracel YM membranes:

- YM-3 (yellow top) — 3,000 Nominal Molecular Weight Limit (NMWL)
- YM-10 (green top) — 10,000 NMWL
- YM-30 (clear top) — 30,000 NMWL
- YM-50 (rose top) — 50,000 NMWL
- YM-100 (blue top) — 100,000 NMWL

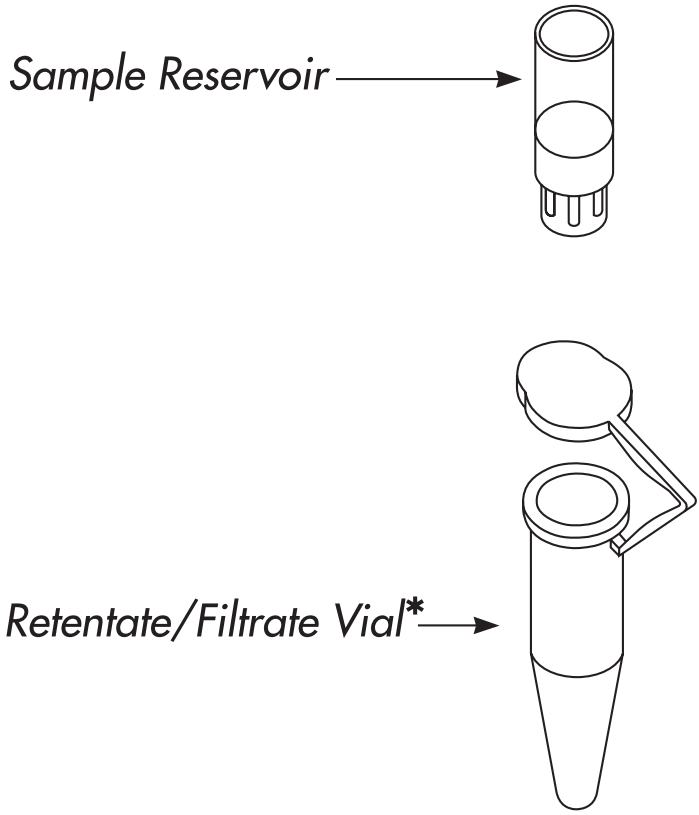
Ultracel low-binding, anisotropic, hydrophilic regenerated cellulose membrane is used in other high recovery centrifugal filter devices including Centricon®, Centricon Plus-20, Centricon Plus-70, Centriprep®, and Centriplus® devices. Because the same membrane is used in many of Millipore's centrifugal filter devices, scale up for higher volumes simply by switching to a larger device.

Introduction, continued

The low-adsorption characteristics of the Ultracel YM membrane and the device's component parts, together with an inverted recovery spin, combine to yield unusually high recovery rates—typically > 95% of the sample, with concentration factors as high as 100×. The results obtained are consistent, assuring easy reproducibility from experiment to experiment.

Typical processing time is just 12–70 minutes. Deadstop prevents spinning to dryness and potential sample loss.

Diagram of Microcon Centrifugal Filter Device



*2 identical vials are supplied with each Microcon unit for use in concentration and recovery spins.

Color codes

Yellow — Ultracel YM-3 membrane
Green — Ultracel YM-10 membrane
Clear — Ultracel YM-30 membrane
Rose — Ultracel YM-50 membrane
Blue — Ultracel YM-100 membrane

Intended Use

Microcon centrifugal filter devices are for research use only, not for use in diagnostic procedures.

Applications

A full range of membrane cut-offs give Microcon centrifugal filter devices great flexibility in performing a variety of functions in addition to standard concentration/desalting operations. Other uses include: buffer exchange; removal of primers, linkers, or molecular labels from a reaction mix; and protein removal prior to HPLC. Microcon centrifugal filter devices provide a fast, efficient means for concentrating RNA/DNA samples, either single- or double-stranded. The nucleotide cut-offs (NCO) listed in the "Centrifugation Guidelines" section can be used as a guide in selecting the appropriate Microcon model for each application.

Equipment Required

Any centrifuge that can properly accommodate 1.5 mL microcentrifuge tubes is acceptable, although fixed-angle rotors are preferred. If using a Microcon device with Ultracel YM-100 membrane to process DNA or RNA, a variable-speed centrifuge is required.

Materials Supplied

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

Limitations

Microcon components are not autoclavable. Do not operate above the following limits. Excessive g-force may result in leakage or damage to the device.

Maximum Centrifugal Force

- Ultracel YM-3, YM-10, YM-30, and YM-50 membrane: 14,000 × g
- Ultracel YM-100 membrane for proteins: 14,000 × g
- Ultracel YM-100 membrane for RNA/DNA samples: 500 × g

Centrifugation Guidelines for Microcon Devices

Ultracel YM Membrane	Color Code	Membrane NMWL	NCO SS	NCO DS	Maximum g-Force	Spin Times* 25 °C**
YM-3	Yellow	3,000	10	10	14,000	100
YM-10	Green	10,000	30	20	14,000	30
YM-30	Clear	30,000	60	50	14,000	12
YM-50	Rose	50,000	125	100	14,000	12
YM-100	Blue	100,000	300	125	14,000	12
					500[1]	12

NMWL: Nominal molecular weight limit in Daltons (proteins)
NCO: Nucleotide cut-off for RNA/DNA (SS = single stranded, DS = double stranded)
*Time in minutes; 500 µL samples reduced to 10 µL.
**Sample concentration using Microcon devices at 4 °C typically takes twice as long as that at 25 °C.
[1]For RNA/DNA samples using Microcon devices with Ultracel YM-100 membrane, the recommended g-force is 500 × g.
NOTE: DNA sample concentration using Microcon devices generally requires shorter spin times. For example, at 25 °C, a 10 µL final volume of an RNA/DNA sample can be achieved in a Microcon device with Ultracel YM-30 membrane in just 8 minutes.

Prerinsing Devices

Microcon ultrafiltration membranes contain trace amounts of glycerin. If glycerin interferes with analysis, spin-rinse devices with deionized water or 0.1 N NaOH until no more interference is detected. If using 0.1 N NaOH, spin-rinse devices with deionized water until pH of filtrate is neutral.

NOTE: Do not allow membranes to dry out before use.

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 sample reservoir, agitate gently for 30 seconds, then proceed with recovery.

Storage

For best results, store all unused Microcon centrifugal filter devices at room temperature.

Chemical Compatibility

The solutions listed in this section have been evaluated for chemical compatibility in Microcon devices containing Ultracel YM membranes. Contact with some organic chemicals may cause leaching from component parts. If leaching is suspected, run solvent blanks before performing assays.

Test conditions: 6-hour exposure at 25 °C.

Common Acids/Alkalis	
Acetic acid (50%)	✓
Ammonium hydroxide (10%)	✓
Formic acid (50%)	✓
Formic acid (70%)	✗
Hydrochloric acid (1.0 N)	✓
Hydrochloric acid (0.01 N)	✓
Lactic acid (50%)	✓
Sodium hydroxide (0.1 N)	✓
Sodium hydroxide (2.5 N)	✗
Sulfuric acid (3%)	✓
Sulfuric acid (30%)	✗
Trichloroacetic acid (10%)	✓
Trifluoroacetic acid (30%)	✓

Organic Solvents/Miscellaneous	
Acetone	✗
Acetonitrile (20%)	✓
Alconox® detergent (1%)	✓
Ammonium sulfate (saturated)	✓
Benzene	✗
Carbon tetrachloride	✗

✓ = Acceptable ✗ = Not Recommended ? = Questionable

Chemical Compatibility, continued

Organic Solvents/Miscellaneous

CHAPS (100 mM)	✓
Chloroform	✗
Diethyl pyrocarbonate (0.2%)	✓
Dimethyl formamide	✗
Dimethyl sulfoxide (5%)	✓
Dioxane	✗
Ethyl acetate	✗
Formaldehyde (5%)	✓
Formamide	✗
Glycerin	✓
Guanidine HCL (6 M)	✓
Guanidine thiocyanate (0.5 M)	✓
Guanidine thiocyanate (5 M)	✗
Lubrol® PX emulsifier (0.1%)	✓
Mercaptoethanol (0.1 M)	✓
Nonidet™ P-40 surfactant (2%)	✓
Phenol (1%)	✓
Phosphate buffer (1 M, pH 8.2)	✓
Polyethylene glycol	✓
Pyridine	✗
Sodium carbonate (20%)	✓
Sodium chloride (2M)	✓
Sodium deoxycholate (5%)	✓
Terg-A-Zyme® detergent (1%)	✓
Tetrahydrofuran	✗
Toluene	✗
Tris buffer (1 M, pH 8.2)	✓
Triton® Z-100 surfactant (5 mM)	✓
Tween®-20 surfactant (0.1%)	✓
Urea (8 M)	✓

✓ = Acceptable ✗ = Not Recommended ? = Questionable

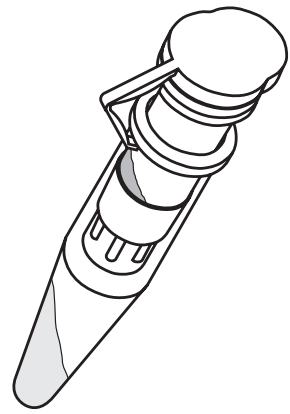
How to Use the Microcon Centrifugal Filter Device

1. Insert Microcon sample reservoir into vial.

2. Pipette solution into sample reservoir (0.5 mL maximum volume), without touching 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.

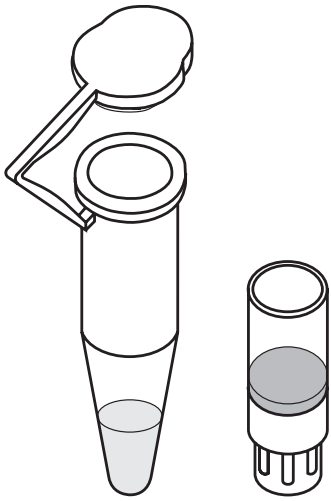


Assembled device during concentration spin

How to Use the Microcon Centrifugal Filter Device, continued

4. Spin using the "Centrifugation Guidelines" for correct spin times and speeds.

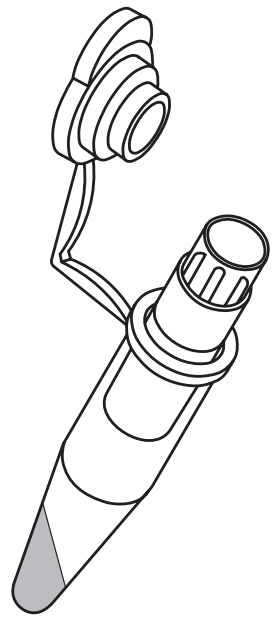
5. Remove assembly from centrifuge. Separate vial from sample reservoir.



Individual components separated after spinning

How to Use the Microcon Centrifugal Filter Device, continued

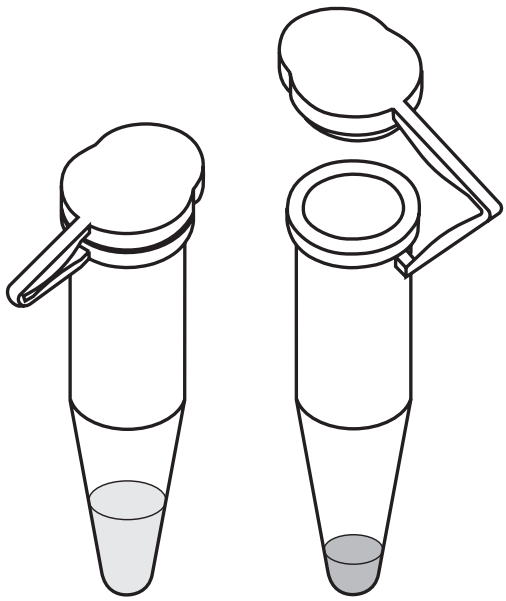
6. Place sample reservoir upside down in a new vial, then spin 3 minutes at 1000 × g (or pulse briefly) to transfer concentrate to vial.



Retentate transfer during recovery spin

How to Use the Microcon Centrifugal Filter Device, continued

7. Remove from centrifuge. Separate sample reservoir. Snap sealing cap shut to store product for later use.



Filtrate and retentate in sealable storage vials

Performance

Tests using Microcon centrifugal filter devices have characterized flow rate, retention and recovery for several well-known materials. These results, summarized in the "Typical Protein Recovery" section, may help when estimating concentrator performance with other solutes. Actual performance, however, depends upon the nature of the specific solute under study.

The centrifugal forces presented here are measured at the base of the filtrate cup. Calculate centrifugal force using this formula:

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

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

Flow Rate

Factors affecting flow rate include solute type and concentration, the starting volume, relative centrifugal force, angle of centrifuge rotor, membrane type (nominal molecular weight limit) and temperature.

Typical Protein Recovery

Solute/Concentration	Nominal MW	% Retentate Recovery by Model Ultracel YM membrane				
		3	10	30	50	100
Bovine IgG Fraction II (1 mg/mL)	156,000	95	95	95	95	95
Bovine serum albumin (1 mg/mL)	67,000	95	95	95	90	50
Ovalbumin (1mg/mL)	45,000	95	95	95	90	10
α-Chymotrypsinogen (1 mg/mL)	25,000	95	95	95	80	2
Cytochrome c (0.25 mg/mL)	12,400	95	95	90	10	1
Protamine sulfate (1 mg/mL)	5,000–10,000	90	20	5	3	1
Vitamin B12 (0.2 mg/mL)	1,355	10	3	1	2	2
Riboflavin (saturated solution)	376	5	2	1	4	1

Retention and Recovery

The anisotropic, hydrophilic Ultracel YM membranes in Microcon centrifugal filter devices are characterized by a nominal molecular weight limit (NMWL), i.e., the ability to retain molecules above a specified molecular weight. While membrane retention is most closely related to molecular size and shape, molecular weight has proven to be a more convenient parameter to use for most applications. The membrane's NMWL is based on results achieved with well-known solutes but may not hold true for all solutes. In particular, solutes with molecular weights close to the membrane cut-off may be partially retained.

Low solute recovery in the retentate may indicate possible adsorptive losses and/or solute passage through the membrane. Adsorptive losses in Microcon devices depend upon several factors including: solute concentration, the hydrophobic nature of the solute, operating temperature, length of time the solute is in contact with component surfaces, sample composition, and pH.

Desalting/Diafiltration

Microcon centrifugal filter devices are useful in a variety of applications, including desalting and buffer or solvent exchange. To remove low molecular weight contaminants using Microcon centrifugal filter devices, concentrate repeatedly, then reconstitute with the appropriate solvent. Typically two spins, each concentrating the sample 20-fold, will provide 95% exchange of buffers or removal of low-molecular-weight contaminants.

How to Quantitate Recoveries

To calculate percent filtrate and retentate recovery, use the formulas below.

$$\% \text{ retentate recovery} = 100 \times \frac{W_r \times C_r}{W_o \times C_o}$$

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

W_r = total weight of retentate before assay

W_o = weight of starting material

W_f = weight of filtrate

C_r = retentate concentration

C_o = starting material concentration

C_f = filtrate concentration

Specifications

Maximum initial sample volume: 500 µL (0.5 mL)

Typical final concentrate volume: 5–15 µL

Maximum relative centrifugal force:

Ultracel YM-3, YM-10, YM-30 & YM-50 membranes: 14,000 × g

Ultracel YM-100 membrane:
14,000 × g for proteins
500 × g for RNA/DNA

Active membrane surface area: 0.32 cm^2

Hold-up volume: 10 µL *(unrecoverable volume trapped beneath membrane)*

Diameter: 12.3 mm

Length, concentration mode: 45.0 mm *(includes concentrator in capped filtrate vial)*

Length, recovery mode: 48.2 mm *(includes concentrator and retentate vial)*

Materials of Construction

Membrane: Ultracel YM regenerated cellulose membrane

Sample reservoir: polycarbonate

Membrane support base: acetal

Filtrate/retentate vial: polypropylene

O-ring: medical-grade silicone rubber

Ordering Information

This section lists the catalogue numbers for Microcon centrifugal filter devices. See the Technical Assistance section for information about contacting Millipore. You can also buy Millipore products on-line at www.millipore.com/purecommerce.

Membrane	NMWL & Color	Units per Pack	Product Number
Ultracel YM-3	3,000 Yellow Top	8	42420
		24	42403
		100	42404
Ultracel YM-10	10,000 Green Top	8	42421
		24	42406
		100	42407
Ultracel YM-30	30,000 Clear Top	8	42422
		24	42409
		100	42410
Ultracel YM-50	50,000 Rose Top	8	42423
		24	42415
		100	42416
Ultracel YM-100	100,000 Blue Top	8	42424
		24	42412
		100	42413

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

Support Documents on the Internet

Millipore maintains a library of current protocol notes and other product information for Microcon centrifugal filter devices on our web site: www.millipore.com/publications.

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