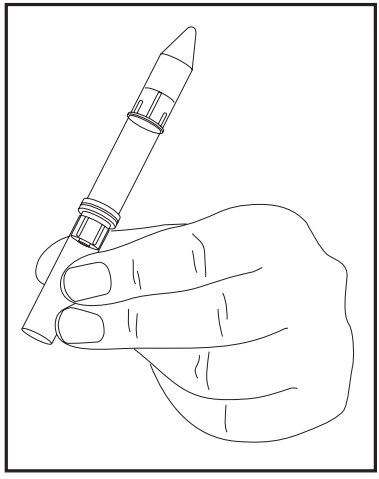
CENTRICON®

Centrifugal Filter Devices

User Guide



- Centricon centrifugal filter devices with 10,000 NMWL, 30,000 NMWL, 50,000 NMWL, and 100,000 NMWL Ultracel® YM membranes are approved for in-vitro diagnostic use.
- Centricon centrifugal filter devices with 3,000 NMWL Ultracel YM membranes are for research use only and are not for use in diagnostic procedures.

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BIOSEPARATIONS

Contents

Introduction

Centricon centrifugal filter devices provide fast, efficient concentration and desalting of macromolecular solutions by ultrafiltration using Millipore's Ultracel® YM regenerated cellulose membranes. Designed for use in centrifuges with fixed-angle rotors, they can provide up to 80-fold sample enrichment with minimal solute loss by adsorption.

The Centricon product line currently includes devices with:

Ultracel YM-3 membrane — 3,000 NMWL

Ultracel YM-10 membrane — 10,000 NMWL

Ultracel YM-30 membrane — 30,000 NMWL

Ultracel YM-50 membrane — 50,000 NMWL

Ultracel YM-100 membrane — 100,000 NMWL

Concentration is achieved by ultrafiltering the sample solution through an anisotropic membrane. Centrifugal force drives solvents and low molecular weight solutes through the membrane into the filtrate vial. Retained macrosolutes are above the membrane inside the sample reservoir. As the sample volume is diminished, retained solute concentration increases. For recovery, the sample is transferred to the retentate vial by placing the vial over the sample reservoir, inverting the device, then centrifuging briefly. This minimizes adsorptive losses to the membrane and the reservoir wall.

Introduction, continued

Centricon centrifugal filter devices are designed for use only in centrifuges with fixed-angle rotors. This results in a force vector that is at a constant angle to the membrane surface. As sample concentration progresses, retained material slides outward and gathers at the edge of the membrane. As a result, the filtration rate remains consistently high. In a swinging-bucket rotor, the horizontal angle would force the retentate layer to accumulate over the entire membrane surface, significantly reducing solvent flow.

The membrane support contains a circular outlet with ducts for solvent flow. Filtration stops when the solvent meniscus reaches the outer edge of the outermost duct. This deadstop prevents concentration to dryness. Rotor angle determines final deadstop volume, as outlined in the "Specifications" section.

Intended Use

Centricon centrifugal filter devices containing Ultracel YM-10, YM-30, YM-50, and YM-100 membranes are for in vitro diagnostic use and are intended for concentrating serum, urine, cerebrospinal fluid and other body fluids before analysis.

Centricon devices containing Ultracel YM-3 membranes are for research use only and are not approved for use in diagnostic procedures.

For in vitro diagnostic use CE Millipore cat. no.			For research use only Millipore cat. no.
4241	4208	4225	4240
4205	4209	4244	4202
4206	4210	4211	4203
4207	4243	4212	
4242AM	4224	4213	

Applications

- Concentration of biological samples containing antigens, antibodies, enzymes, nucleic acids, microorganisms and other macromolecular material.
- Purification of macromolecular components found in tissue culture extracts and cell lysates.
- Recovery of oligonucleotides and peptides.
- Filtration of substances with MW below the nominal molecular weight limit (NMWL).

Equipment Required

Any centrifuge with fixed-angle rotor adaptors or carriers accepting 17 × 100 mm tubes and capable of 1000–7500 × g. Millipore recommends using a covered rotor.

Usage Guidelines

Centricon 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

- Centricon filter devices with Ultracel YM-3 membranes:
  without retentate vial: 6500 × g
  with retentate vial: 7500 × g
  during prerinsing: 5000 × g
- Centricon filter devices with
  Ultracel YM-10, YM-30, and YM-50 membranes: 5000 × g
- Centricon filter devices with Ultracel YM-100 membranes:
  5000 × g for proteins;
  2000 × g for nucleic acids (adjust the sample to 2 mL with TE or other appropriate buffer prior to centrifugation)

Rotor Compatibility

Visit www.millipore.com/rotorguide for centrifuge and rotor compatibility for Millipore centrifugal separation devices.

To Convert Relative Centrifugal Force (RCF) to Revolutions Per Minute (RPM):

Determine the centrifuge's radius of rotation by measuring the distance from the center of the centrifuge spindle to the bottom of the device when it is inserted into the rotor.

Relative centrifugal force can be calculated using the formula:

$$RCF = 1.118 \times 10^{-5} \times \text{radius} \times (RPM)^2$$

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

RPM = revolutions per minute

Prerinsing

The ultrafiltration membranes in Centricon centrifugal filter devices contain trace amounts of glycerine (approx. 2 µL per device).

If these materials interfere with analysis, fill the device with 2 mL of buffer or deionized water and spin until at least half the rinse passes into the filtrate vial then invert the device and spin at 300–1000 × g for two minutes to remove the remaining rinse. If interference continues, repeat with a second spin of buffer or deionized water.

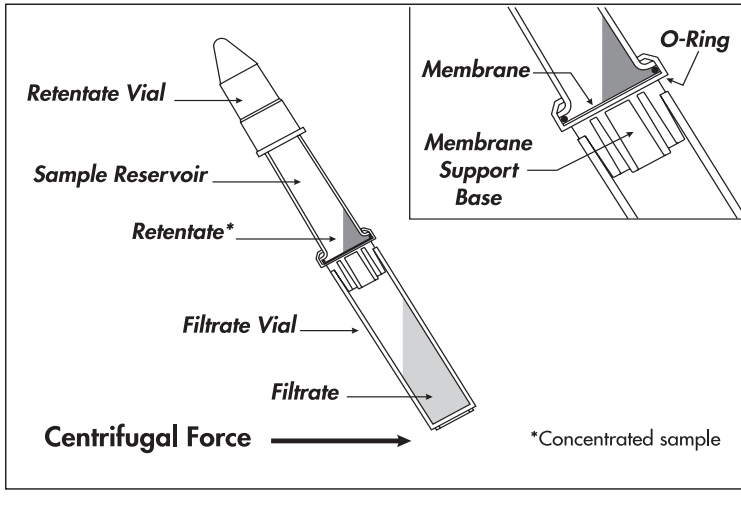
CAUTION: Do not allow the membrane in Centricon filter devices to dry out once wet. If you are not using the device immediately after prerinsing, leave fluid on the membrane until the device is used.

Chemical Compatibility

Centricon centrifugal filter devices are intended for use with biological fluids and aqueous solutions. Before use, check the sample for chemical compatibility with the device. The chemical compatibility list can be found at www.millipore.com/centrifugal.

How to Use Centricon Centrifugal Filter Devices

1. Insert sample reservoir into filtrate vial as shown.



2. Add solution to sample reservoir (2 mL maximum volume). Do not touch membrane with pipette tip. If possible, use a covered rotor in order to minimize sample evaporation. Or, seal the device by attaching the retentate vial to the sample reservoir. The rotor adapter must accept a minimum length of 137 mm.

   CAUTION: Obstructions could damage the centrifugal device during centrifugation. Check clearance.

3. Place covered device and attached filtrate vial into the centrifuge rotor; counterbalance with a similar device.

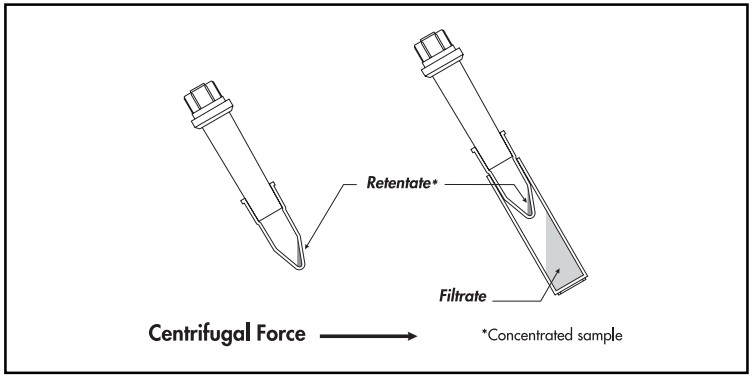
How to Use Centricon Centrifugal Filter Devices, continued

4. Spin as follows until desired concentration is achieved:
   Centricon devices with Ultracel YM-3 membrane:
   4000–7500 × g with retentate vial in place;
   4000–6500 × g without retentate vial
   Centricon devices with Ultracel YM-10, YM-30, and YM-50 membranes: 1000–5000 × g
   Centricon devices with Ultracel YM-100 membrane:
   5000 × g for proteins;
   2000 × g for nucleic acids (adjust the sample to 2 mL with TE or other appropriate buffer prior to centrifugation)

   CAUTION: Do not exceed the recommended centrifugation speed.

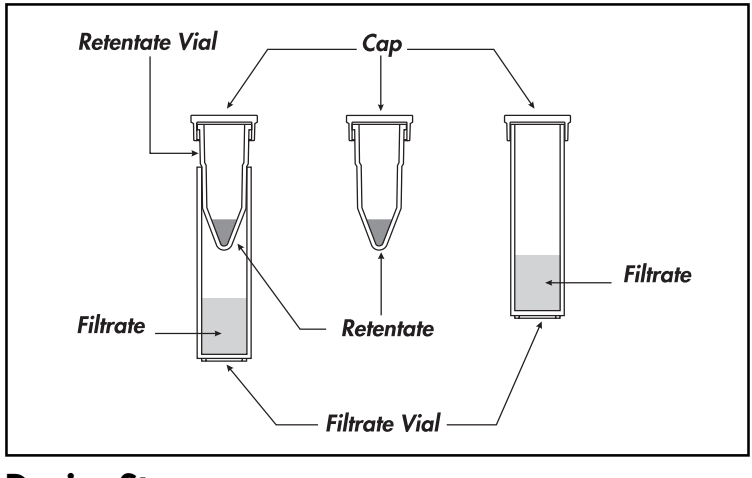
5. Remove centrifugal filter assembly from centrifuge; then separate filtrate vial from membrane support base. For filtration applications, reserve filtrate. For concentration applications, filtrate may either be discarded or left in the filtrate vial.

6. Place retentate vial over sample reservoir as shown and invert unit to recover the retentate. Centrifuge at 300–1000 × g for two minutes to transfer concentrate into retentate vial.



How to Use Centricon Centrifugal Filter Devices, continued

7. Remove device from centrifuge. Separate retentate vial from concentrator. Use the supplied cap to cover retentate or filtrate vial. You can use the retentate vial to cover the filtrate vial, if you want. This permits storage of retentate and filtrate together in one place.



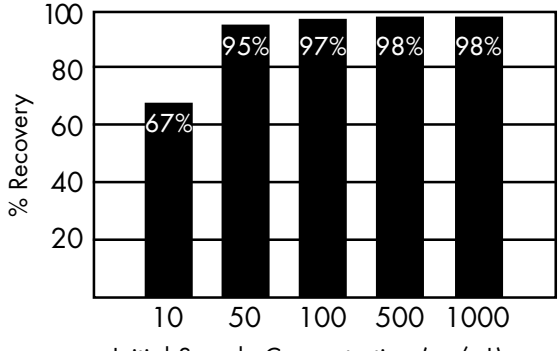
Device Storage

For best results, store between 15 °C and 30 °C. Storage below 0 °C or above 40 °C may affect performance.

Performance

Flow rate, retention, and recovery have been established with well-known materials. Use the data in the "Recovery vs. Sample Concentration, Cytochrome c" and "Typical Retentate Recovery" charts to estimate performance with other solutes. Actual performance depends upon the specific concentration and solute used.

Recovery vs. Sample Concentration, Cytochrome c



2.5 hour spin at 7,500 x g, 3,000 NMWL Centricon device

Flow Rate

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

Typical Retentate Recovery

Solute/Concentration	MW	% Retentate Recovery by Model Ultracel YM 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	95	85
Ovalbumin (1 mg/mL)	45,000	95	95	95	85	40
α-Chymotrypsinogen (1 mg/mL)	25,000	90	95	90	75	≤ 5
Cytochrome c (0.25 mg/mL)	12,400	95	95	85	15	< 1
Protamine Sulfate (1 mg/mL)	5,000–10,000	80	10	≤ 5	≤ 5	≤ 5
Vitamin B12 (0.2 mg/mL)	1,355	15	≤ 5	≤ 5	≤ 5	≤ 5
Riboflavin (saturated solution)	376	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5

Spin Times: *For spin times, refer to the "Centrifugation Guidelines" chart.*

Retention and Recovery

The membranes used in Centricon devices are characterized by a nominal molecular weight limit; that is, their ability to retain molecules above a specified molecular weight. Solutes with molecular weights close to the membrane cut-off may only be 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. For applications involving nucleic acids, strand length is the most useful parameter for selecting the Centricon device appropriate for a particular application. However, other parameters including DNA concentration (see "Nucleic Acid Recovery Chart"), the magnitude of the driving force (g-force) and the salt concentration all act in concert to affect DNA recovery.

When purifying PCR reactions for example, optimal yield can be achieved by diluting the starting material and running at a reduced g-force. For PCR fragments <1000 bp in length, using a Centricon-50 device will generally result in the best balance between speed and recovery. Similarly, for DNA fragments >1000 bp, choose a Centricon-100 device, as shown in "Centrifugation Guidelines". If the DNA sample is in a buffer containing high salt and cannot be diluted, or the device is run at a higher than recommended g-force, a significantly reduced DNA recovery will likely be observed. Under these conditions, higher DNA or RNA recovery can be achieved by using the Centricon device with the next tighter membrane, albeit with a much longer processing time. Note that, for maximal removal of unincorporated single-stranded primers from double-stranded DNA fragments, the molecular weights of the primer and DNA fragment should differ by at least an order of magnitude.

Nucleic Acid Recovery

Nucleic Acid	Initial Sample Concentration 10 ng/mL	40 ng/mL	400 ng/mL	4 µg/mL
DNA[1]	97%	98%	99%	99%
RNA[2]	98%	96%	99%	98%
Oligomer[3]	87%	90%	90%	90%

[1] pBR322, 4.3 kilobases [2] pSPT18-neo-DNA transcript, 1.0 kilobase [3] 25 bases

Operating procedure:

^{32}P-labeled DNA was concentrated in 250 µL of 0.3 M sodium acetate buffer, using a Centricon-30 device (10-minute spin at 5,000 × g).

^{32}P-labeled RNA and oligomer were concentrated in 250 µL of TE buffer (10 mM Tris, pH 8, 1 mM EDTA), using a Centricon-30 and Centricon-3 device, respectively.

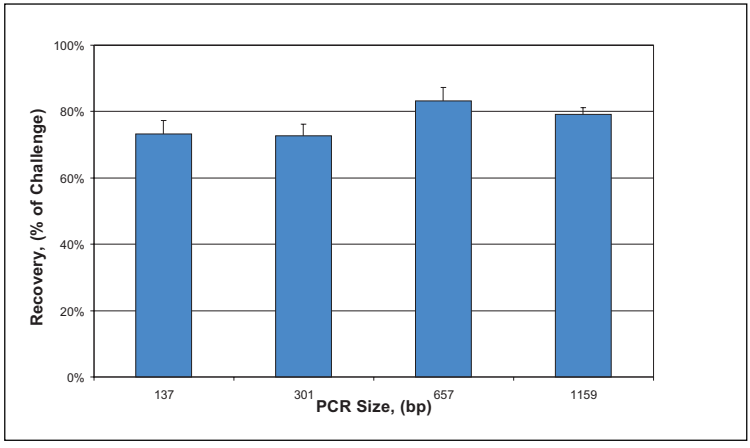
Sample recovery was determined by counting Cherenkov radiation.

Centrifugation Guidelines for Nucleic Acids Using Centricon Devices

Membrane	Base Color Code	Membrane NMWL	DNA Fragment Size	Spin Times* 25 °C	Maximum g-Force
Ultracel YM-3	Yellow	3,000	Small Oligos, > 10 bases	2 hours	7,500**
Ultracel YM-10	Green	10,000	Oligos, > 30 bases	1 hour	5,000
Ultracel YM-30	Clear	30,000	Oligos, > 50 bases	30 min	5,000
Ultracel YM-50	Rose	50,000	125–1,000 bp	15 min	5,000
Ultracel YM-100	Blue	100,000	> 1,000 bp, genomic DNA	10 min	2,000

NMWL: Nominal Molecular Weight Limit
*2 mL samples
5,000 during prerinsing procedure

Purification of PCR Fragments Using Centricon Device, 50,000 NMWL



For purification of DNA fragments generated by PCR, the 100 µL reactions were diluted with 2.0 mL of TE Buffer (10 mM Tris-HCL, pH 8.0, 1 mM EDTA) and centrifuged at 5,000 × g for 15 minutes. The sample reservoir was filled with another 2.0 mL of TE buffer and centrifuged again as above. The purified PCR products were collected by a reverse spin and the recovered DNA quantified by a fluorometric assay.

Biological Activity

In lab tests, 1 mL of lactic dehydrogenase (type 5S rabbit muscle, M4 isoenzyme, 99% homogenous) containing 5 units of enzyme activity and 1 mg/mL bovine serum albumin (to minimize nonspecific adsorption) was spun in a Centricon-30 device for 30 minutes at 2000 × g. All five units of the original activity were recovered with 90% recovery of bovine serum albumin.
[A unit = amount of activity to reduce 1 µmole of pyruvate to lactate per minute at pH 7.5, 37 °C.]

Low Solute Recovery

Low solute recovery in the retentate may indicate adsorptive losses and/or passage of solute through the membrane. Adsorptive losses depend upon solute concentration, its hydrophobic nature, temperature and time of contact with component surfaces, sample composition and pH. If retentate (i.e., concentrate) recoveries are lower than expected, reconcentrate filtrate with a fresh device.

Membrane Appearance

After centrifugation, the membrane surface may wrinkle slightly. This is normal due to membrane expansion in water and subsequent flattening by pressure. Concentrator performance is unaffected. The membrane's plasticity prevents the creases from becoming cracks.

Desalting

Desalting or solvent exchange is accomplished by concentrating the sample, then reconstituting the retentate to the original sample volume with any desired solvent. Repeat this process until the concentration of the contaminating microsolute has been sufficiently reduced.

DNA Desalting, Centricon Device, 30,000 NMWL

Spin No.	CsCl Concentration	DNA Recovery
1	510 mM	94%
2	58 mM	91%
3	7 mM	96%
4	0.8 mM	97%

Operating procedure: 1.0 mL of 25 µg/mL *E. coli* DNA in 6M CsCl was repeatedly concentrated to 0.1 mL. Sample was spun at 2000 × g in a centrifuge with a 45° fixed-angle rotor. Concentrate was reconstituted to original volume by adding 50 mM Tris. After four spins, CsCl concentration was reduced by four orders of magnitude.

Important note: When desalting or purifying small volumes (i.e., 50–250 µL); dilute the sample to 2 mL before spinning. Often this will reduce salts or primers to an acceptable level in a single spin. (Example: If a 100 µL sample is diluted to 2 mL, then concentrated to 25 µL, over 98% of the salt will be removed.)

Specifications

Maximum initial sample volume:	2.0 mL
Typical final concentrate volume:	45° angle rotor: approx. 25 µL 34° angle rotor: approx. 40 µL 28° angle rotor: approx. 50 µL
Filtrate vial capacity:	45° angle rotor: 3.4 mL 34° angle rotor: 3.1 mL 28° angle rotor: 2.9 mL
Retentate vial capacity:	45° angle rotor: 0.7 mL 34° angle rotor: 0.6 mL 28° angle rotor: 0.5 mL

Maximum relative centrifugal force:

- Centricon devices with Ultracel YM-3 membrane: 7500 × g
- Centricon devices with Ultracel YM-10, YM-30, and YM-50 membranes: 5000 × g
- Centricon devices with Ultracel YM-100 membrane:

  For DNA concentration: 2000 × g (adjust the sample to 2 mL with TE or other appropriate buffer prior to centrifugation)

  For protein concentration: 5000 × g

Active membrane area: 0.92 cm^2

Hold-up volume of membrane and support: 0.02 mL

Specifications, continued

Dimensions: Diameter: 16 mm

Length, concentration mode: 126–137 mm
(retentate vial, concentrator and filtrate vial)

Length, concentration mode: 101–107 mm
(concentrator and filtrate vial only)

Length, recovery mode: 115–117 mm
(concentrator, retentate vial and filtrate vial)

Length, recovery mode: 82–88 mm
(concentrator and retentate vial only)

Materials of Construction

Sample reservoir: styrene acrylonitrile

Membrane support base: polycarbonate

O-ring: red silicone rubber

Filtrate and retentate vials, cap: polypropylene

Membrane material: Ultracel YM regenerated cellulose membrane

Product Ordering Information

This section lists the catalogue numbers for Centricon 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	Color Code	NMWL	Units/Pkg.	Cat. No.
Ultracel YM-3	yellow	3,000	8 24 100	4240 4202 4203
Ultracel YM-10	green	10,000	8 24 100 500	4241 4205 4206 4207
Ultracel YM-30	clear	30,000	8 24 100 500	4242AM 4208 4209 4210
Ultracel YM-50	rose	50,000	8 24 100	4243 4224 4225
Ultracel YM-100	blue	100,000	8 24 100 500	4244 4211 4212 4213
Components*				
Filtrate vials Retentate vials Caps			100 100 100	4215 4217 4219

**Included in all packages, one per Centricon unit*

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 http://www.millipore.com/techservice.

For matters associated with Directive 98/79/EC on in vitro diagnostic medical devices, contact our European Representative:

Millipore Ireland BV,
Tullagreen, Carrigtwohill, County Cork
Ireland

Support Documents on the Internet

Millipore maintains a library of current protocol notes and other product information for Centricon Centrifugal Devices on the Internet at our World Wide Web site: http://www.millipore.com/publications. A user interface appears, allowing you to enter search criteria. Select **Centricon**. A series of links appears, each to an online product publication.

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