User Guide

Microcon® Centrifugal Filter Devices

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

Introduction

Microcon® centrifugal filters provide efficient concentration, desalting, or buffer exchange of aqueous biological samples ranging in volume from 10–500 µL. The low-adsorption characteristics of the Ultracel® membrane and device's component parts, plus an inverted recovery spin, combine to yield unusually high recovery rates. 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® 10K and 30K devices are provided non-sterile, and Microcon® DNA Fast Flow devices are available in either the standard non-sterile format, or in a PCR grade dual-cycle ethylene oxide (2xEtO) treated format. This 2xEtO treatment does not remove contaminant DNA, but has been shown to sufficiently fragment DNA so that it is not PCR-amplifiable.[1, 2]

The Microcon® product line includes:

- Microcon® 10K device (green top)
- Microcon® 30K device (clear top)
- Microcon® DNA Fast Flow device (blue top)

  Standard non-sterile **and** PCR grade (2xEtO) formats

Intended Use

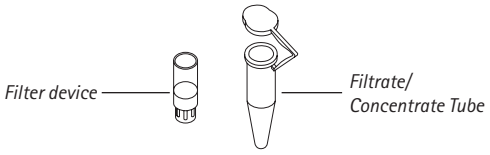
Microcon® centrifugal filter devices are for research use only. They are not for use in diagnostic procedures.

Applications Guidelines

Use the following table to choose the correct Microcon® device for your application.

Microcon® Device	10K	30K	DNA Fast Flow
Peptide and growth factor concentration	●		
Protein concentration and desalting of columns eluates	●	●	
Protein concentration before electrophoresis or other assays	●	●	
Protein removal prior to HPLC	●	●	
Purification of macromolecular components found in tissue culture extracts and cell lysates	●	●	
Concentration of biological samples (antigens, antibodies, enzymes)		●	
Concentration and desalting of nucleic acids (single-or double-stranded)	●	●	●
Removal of labeled nucleotides	●	●	●
Removal of labeled amino acids	●	●	●
Removal of primers from amplified DNA		●	●
Removal of linkers prior to cloning		●	●

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 micro-centrifuge tubes is acceptable, although fixed-angle rotors are preferred. A variable speed centrifuge is required for Microcon® DNA Fast Flow devices.

Suitability

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

Microcon® DNA Fast Flow devices are particularly suitable for concentrating DNA samples in simple buffers such as Tris-EDTA (TE), or DNA samples extracted with Phenol/Chloroform/Isoamyl alcohol (PCI).

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® Device	G-force
10K	14,000 × g
30K	14,000 × g
DNA Fast Flow	500 × 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

The ultrafiltration membranes in Microcon® devices contain trace amounts of glycerine. If this material interferes with analysis, rinse the device with buffer or distilled water before use. If interference continues, rinse with 0.1 N NaOH followed by a second spin of buffer or distilled water.

When rinsing Microcon® DNA Fast Flow PCR Grade devices, use aseptic technique when handling devices, and use sterile, nuclease-free and nucleic acid-free buffers and reagents.

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® Filter Device

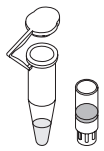
NOTE: For Microcon® DNA Fast Flow PCR Grade devices, use aseptic technique when opening packages and throughout the procedure. Carefully reseal pouches to protect unused samples from contamination.

1. Insert Microcon® 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.

Assembled device during concentration spin

4. Spin at 14,000 x g for Microcon® 10K and 30K devices and 500 x g for DNA Fast Flow devices. Refer to Table 1 for typical spin times.
5. Remove assembly from centrifuge. Separate tube from filter device.

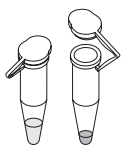


Individual components separated after spinning

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.

Concentrate transfer during recovery spin

7. Remove from centrifuge. Separate tube from filter device. Close sealing cap to store sample for later use.



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® 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 microsolute 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.

NOTE: Multiple washes may reduce final DNA recovery in Microcon® DNA Fast Flow devices.

Performance

Microcon® 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.

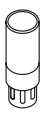
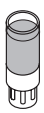
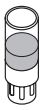
Table 1. Typical Spin Times and Concentration Factors*

Application	Device	G-force (x g)	Typical Spin Time (minutes)	Target Concentration Factor
Protein	10K	14,000	20–40	20–100
Protein	30K	14,000	10–20	20–100
DNA	DNA Fast Flow	500	10–20	≤ 20

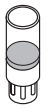
* These guidelines are for starting volumes of 500 µL. For starting volumes less than 500 µL, spin times will be shorter.

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.

Protocol for Optimizing Spin Time		Example
1. Pre-weigh an empty Microcon® 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)

Spin Time Optimization, continued

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.
6. Optimization of the wash spin times may also be done as described above.		

Retention and Recovery

The anisotropic, hydrophilic Ultracel® membranes in Microcon® centrifugal filter devices are characterized by either a molecular weight cutoff (MWCO) or a performance-specific application (e.g., DNA recovery). 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 times smaller than the molecular weight of the protein solute that one intends to concentrate.

Table 2. Typical Protein Recovery Microcon® 10K and 30K Devices

		Typical % Recovery from Concentrate	
Solute/Concentration	Molecular Weight	10K Device	30K Device
Bovine IgG Fraction II (1 mg/mL)	156,000	95	95
Bovine serum albumin (1 mg/mL)	67,000	95	95
Ovalbumin (1 mg/mL)	45,000	95	95
α-Chymotrypsinogen (1 mg/mL)	25,000	95	95
Cytochrome c (0.25 mg/mL)	12,400	95	90
Protamine sulfate (1 mg/mL)	5,000–10,000	20	5
Vitamin B12 (0.2 mg/mL)	1,355	3	1
Riboflavin (saturated solution)	376	2	1

Table 3. Typical DNA Recovery Microcon® DNA Fast Flow Devices*

		Typical % DNA Recovery from Concentrate After Washes			
DNA Sample/Concentration	Buffer Type	0	1	2	3
Human genomic DNA (0.2 µg/mL)	1	85	79	66	64
Human genomic DNA (0.2 µg/mL)	2	94	83	77	75

*** 500 µL starting volume**

Buffer Type 1: Genomic DNA spiked into TE buffer, pH 8.0 and processed with 0 to 3 washes of TE buffer (0 wash = 15 minute spin, and washes 1, 2, and 3 = 13 minute spins each).

Buffer Type 2: Genomic DNA spiked into a "simulated" forensic buffer (10 mM Tris-HCl, pH 8/10 mM EDTA/100 mM NaCl/2% SDS) and extracted using phenol chloroform isoamyl alcohol (25:24:1). Extracted sample loaded and processed with 0 to 3 washes of TE buffer (0 wash = 15 minute spin, and washes 1, 2, and 3 = 13 minute spins each).

Maximizing Sample Recovery

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

Maximizing Sample Recovery, continued

- 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® device.
- For Microcon® 10K and 30K devices in concentration and desalting applications, greater than 95% recovery can be achieved with concentration factors of 100X. For Microcon® DNA Fast Flow devices in DNA applications, the most consistent and reproducible results are achieved when starting volumes are 100–500 µL, and the concentration factor is less than 20X. Recoveries at less than 20X concentration are typically greater than 80%.

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:

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

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

$$\% \text{ total recovery} = \% \text{ concentrate recovery} + \% \text{ filtrate recovery}$$

W_c = total weight of concentrate before assay
W_o = weight of original starting material
W_f = weight of filtrate
C_c = concentrate concentration
C_o = original starting material concentration
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® 10K devices	14,000 × g
Microcon® 30K devices	14,000 × g
Microcon® DNA Fast Flow devices:	500 × 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	Ultracel® low binding regenerated cellulose
Device top	Polycarbonate
Membrane support base	Acetal
Filtrate/concentrate tube	Polypropylene
O-ring	Medical-grade silicone rubber
Device Treatment	
Microcon® DNA Fast Flow device (cat. no. MRCFOR100ET) only	Dual-cycle ethylene oxide (EtO)

Chemical Compatibility

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

Acids	Concentration		Concentration
Acetic acid	≤ 50%	Sulfuric acid	≤ 3%
Formic acid	≤ 50%	Trichloroacetic acid (TCA)	≤ 10%
Hydrochloric acid	≤ 1.0 N	Trifluoroacetic acid (TFA)	≤ 30%
Lactic acid	≤ 50%		

Alkalis			
Ammonium hydroxide	≤ 10%	Sodium hydroxide	≤ 0.1 N

Detergents			
Alconox® detergent	≤ 1%	Sodium deoxycholate	≤ 5%
CHAPS detergent	≤ 100 mM	Tergazyme® detergent	≤ 1%
Lubrol® PX detergent	≤ 0.1%	Triton® X-100 surfactant	≤ 5 mM
Nonidet™ P-40 surfactant	≤ 2%	Tween® 20 surfactant	≤ 0.1%

Organic Solvents	Concentration		Concentration
Acetone	not recommended	Ethyl acetate	not recommended
Acetonitrile	≤ 20%	Formaldehyde	≤ 5%
Benzene	not recommended	Formamide	not recommended
Carbon tetrachloride	not recommended	Isoamyl alcohol*	≤ 1%
Chloroform*	≤ 1%	Pyridine	not recommended
Dimethyl formamide	not recommended	Tetrahyrofuran	not recommended
Dimethyl sulfoxide	≤ 5%	Toluene	not recommended
Dioxane	not recommended		

Miscellaneous			
Ammonium sulfate	Saturated	Phosphate buffer (pH 8.2)	≤ 1 M
Diethyl pyrocarbonate	≤ 0.2%	Polyethylene glycol	≤ 10%
Glycerine	≤ 70%	Sodium carbonate	≤ 20%
Guanidine HCl	≤ 6 M	Sodium chloride	≤ 2 M
Guanidine thiocyanate	≤ 0.5 M	Tris buffer (pH 8.2)	≤ 1 M
Mercaptoethanol	≤ 0.1 M	Urea	≤ 8 M
Phenol	≤ 1%		

* Not compatible with Microcon® 10K and 30K devices

Troubleshooting/Optimization

Problem: Protein recovery in eluted 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 such 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® device was used.
Problem: DNA recovery in eluted fraction is low	
Possible Cause	**Solution**
Over concentration, low concentrate volume	Reduce centrifugation time. Refer to **Spin Time Optimization** section. Reduce centrifugation speed. Microcon® DNA Fast Flow Devices should be spun at 500 × g. Add 10–20 µL of water or buffer prior to recovery spin. Vortex for 10–30 seconds or incubate for 10–30 minutes with gentle mixing prior to recovery spin.
Concentration of DNA in the initial sample was low.	An initial sample mass of less than 100 ng (e.g., 500 µL of 0.2 µg/mL) may not yield as high a recovery as an initial sample mass with a higher DNA concentration.
Volume of the initial sample was low (e.g. < 50 µL).	Optimize centrifugation time. Dilute sample to maximum device capacity (0.5 mL). If buffer exchange is desired, this dilution can be considered the first step of the buffer exchange process. Refer to **Desalting/Diafiltration** section.
Problem: 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 binding buffer.
Cell lysate was too viscous.	Dilute lysate in binding buffer. Include Benzonase® nuclease in lysis buffer to remove free RNA/DNA.

Troubleshooting, continued

Problem: 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.
Problem: 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.
Problem: Sample is contaminated with interfering DNA	
Possible Cause	**Solution**
Aseptic technique was not used.	Use aseptic technique when handling Microcon® components and preparing samples/reagents that come in contact with the device.
The incorrect Microcon® DNA Fast Flow device was used.	Use the Microcon® DNA Fast Flow PCR Grade device, rather than the standard Microcon® DNA Fast Flow device.

References

1. Shaw, K., I. Sesardić, N. Bristol, C. Ames, K. Dagnall, C. Ellis, F. Whittaker, and B. Daniel. 2008. Comparison of the effects of sterilisation techniques on subsequent DNA profiling. *Int J Legal Med* 122:29–33.
2. Archer, E., H. Allen, A. Hopwood, and D. Rowlands. 2010. Validation of a dual cycle ethylene oxide treatment technique to remove DNA from consumables used in forensic laboratories. *Forensic Science International: Genetics* 4:239–243.

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 www.millipore.com/products.

Description	Qty/pk	Catalogue Number
Microcon® 10K Device	100	MRCPRT010
Microcon® 30K Device	100	MRCF0R030
Microcon® DNA Fast Flow Device	100	MRCF0R100
Microcon® DNA Fast Flow PCR Grade Device	20	MRCF0R100ET
Benzonase® Nuclease, Purity >99%	10 KU	70664-3
Benzonase® Nuclease HC, Purity >99%	25 KU	71206-3
Benzonase® Nuclease, Purity >90%	2.5 KU	70746-4
Benzonase® Nuclease, Purity >90%	10 KU	70746-3
Benzonase® Nuclease HC, Purity >90%	25 KU	71205-3

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

Technical Assistance

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

Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms (Conditions of Sale").

Made in Ireland

Merck Millipore Ltd. (Cork, Ireland), an affiliate of Merck KGaA, Darmstadt, Germany
Tullagreen,
Carrigtwohill,
Co. Cork, IRL

PR04305, Rev. 06/18