User Guide

Amicon® Ultra-4 Centrifugal Filter Devices

for volumes up to 4 mL

Amicon® Ultra-4 3K, 30K, 50K, and 100K devices for research use only; not for use in diagnostic procedures

Introduction

Amicon® Ultra-4 centrifugal filter devices provide fast ultrafiltration, with the capability for high concentration factors and easy concentrate recovery from dilute and complex sample matrices. The vertical design and available membrane surface area provide fast sample processing, high sample recovery (typically greater than 90% of dilute starting solution), and the capability for 80-fold concentration. Typical processing time is 10 to 40 minutes depending on Molecular Weight Cutoff (MWCO). Solute polarization and subsequent fouling of the membrane are minimized by the vertical design, and a physical deadstop in the filter device prevents spinning to dryness and potential sample loss. The concentrate is collected from the filter device sample reservoir using a pipettor, while the ultrafiltrate is collected in the provided centrifuge tube. The device can be spun in a swinging-bucket (for optimal performance) or fixed-angle rotor. Amicon® Ultra-4 devices are supplied nonsterile and are for single use only.

The Amicon® Ultra-4 product line includes 5 different cutoffs (MWCO):

- Amicon® Ultra 3K device — 3,000 MWCO
- Amicon® Ultra 10K device — 10,000 MWCO
- Amicon® Ultra 30K device — 30,000 MWCO
- Amicon® Ultra 50K device — 50,000 MWCO
- Amicon® Ultra 100K device — 100,000 MWCO

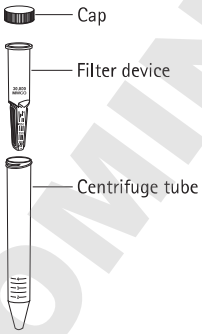
NOTE: The Amicon® Ultra 10K device (10,000 MWCO) is the only device intended for in vitro diagnostic use. For information on this device, go to www.millipore.com/centrifugal_ivd_userguide.

Applications

- Concentration of biological samples containing antigens, antibodies, enzymes, nucleic acids (DNA/RNA samples, either single- or double-stranded), microorganisms, column eluates, and purified samples
- Purification of macromolecular components found in tissue culture extracts and cell lysates, removal of primer, linkers, or molecular labels from a reaction mix, and protein removal prior to HPLC
- Desalting, buffer exchange, or diafiltration

Materials Supplied

The Amicon® Ultra-4 device is supplied with a cap, a filter device, and a centrifuge tube.



Required Equipment

- Centrifuge with swinging-bucket (preferred) or fixed-angle rotor with wells/carriers that can accommodate 17 mm × 124 mm 15 mL conical-bottomed tubes

  CAUTION: To avoid damage to the device during centrifugation, check clearance before spinning.
- Pipettor with 200 microliter (µL) tip for concentrate recovery

Suitability

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

Rinsing Before Use

The ultrafiltration membranes in Amicon® Ultra-4 devices contain trace amounts of glycerine. If this material interferes with analysis, rinse the device with buffer or Milli-Q® water before use. If interference continues, rinse with 0.1 N NaOH followed by a second spin of buffer or Milli-Q® water.

CAUTION: Do not allow the membrane in Amicon® Ultra 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 Amicon® Ultra-4 Centrifugal Filter Devices

1. Add up to 4 mL of sample (3.5 mL if using a 23° fixed-angle rotor) to the Amicon® Ultra filter device.
2. Place capped filter device into centrifuge rotor (swinging-bucket preferred); counterbalance with a similar device.
3. **When using a swinging-bucket rotor,** spin the device at 4,000 × g maximum for approximately 10–40 minutes.

   When using a fixed-angle rotor, orient the device with the membrane panel facing up.

   For Amicon® Ultra 3K, 10K, 30K, and 50K devices, spin at 7,500 × g maximum for approximately 10–40 minutes.

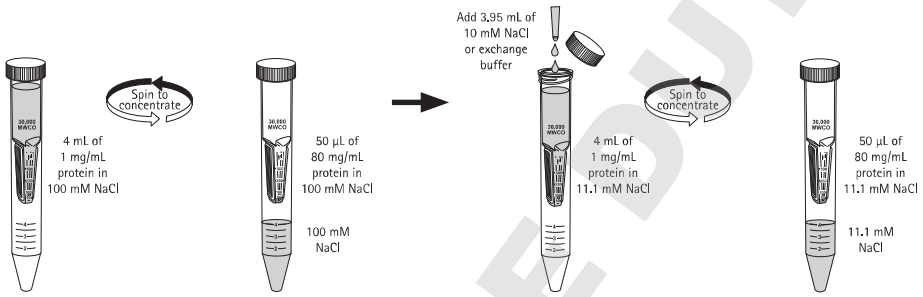
   For Amicon® Ultra 100K devices, spin at 5,000 × g maximum for approximately 10–20 minutes.

   NOTE: Refer to Figures 1 and 2, and Tables 2 and 3 for typical spin times.
4. To recover the concentrated solute, insert a pipettor into the bottom of the filter device and withdraw the sample using a side-to-side sweeping motion to ensure total recovery. The ultrafiltrate can be stored in the centrifuge tube.

   NOTE: For optimal recovery, remove concentrated sample immediately after centrifugation.

Desalting or 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 Amicon® Ultra-4 device by concentrating the sample, 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. See example below.



Performance - DNA Concentration

We have determined that the Amicon® Ultra-4 30K device provides the best balance between recovery and spin time for double-stranded DNA for base pairs ranging from 137 to 1,159.

Table 1. Typical Recovery of Nucleotides from Amicon® Ultra-4 30K Device

Double-stranded DNA Base Pair Size	Spin Time (min)	Concentrate Volume (µL)	Recovery (%)
137–1,159	10	50–70	>85

Spin conditions: Fixed-angle rotor, 5,000 × g, room temperature, 2 mL starting volume.

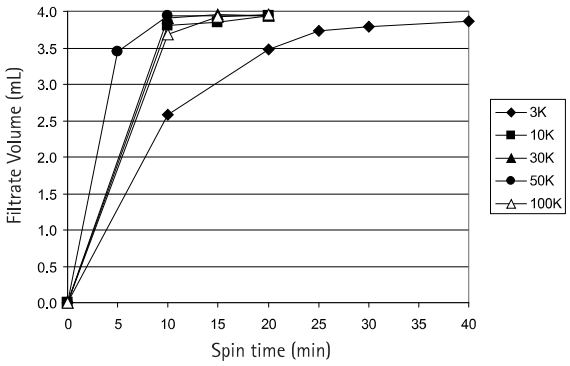
Performance - Protein Concentration

Flow Rate

Factors affecting flow rate include sample concentration, starting volume, chemical nature of solute, relative centrifugal force, centrifuge rotor angle, membrane type, and temperature. Figures 1 and 2, and Tables 2 and 3 can be used to estimate the time required to achieve a given volume of filtrate or concentrate for a variety of protein markers. A typical spin time for a 4 mL sample is approximately 10 to 40 minutes (depending on device molecular weight cutoff). While most of the sample is filtered in the first 10 to 20 minutes of centrifugation, the lowest concentrate volume (30–75 µL) is reached after spinning for 20 to 40 minutes.

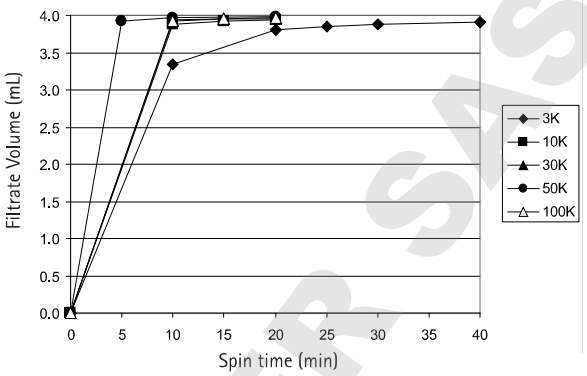
Flow Rate, continued

Figure 1. Typical Filtrate Volume vs. Spin Time (Swinging-bucket rotor)



Spin conditions: 4,000 × g, room temperature, 4 mL starting volume.
Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=6.

Figure 2. Typical Filtrate Volume vs. Spin Time (Fixed-angle rotor)



Spin conditions: 7,500 × g for 3K, 10K, 30K, and 50K, 5,000 × g for 100K, room temperature, 4 mL starting volume. Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=6.

Table 2. Typical Concentrate Volume vs. Spin Time (Swinging-bucket rotor)

	Concentrate volume (µL)				
Spin time (min)	3K device	10K device	30K device	50K device	100K device
10	1,369	176	73	32	264
15	–	76	46	–	36
20	478	58	37	30	33
25	228	–	–	–	–
30	159	–	–	–	–
40	94	–	–	–	–

Spin conditions: 4,000 × g, room temperature, 4 mL starting volume.
Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=6 (mean value of 3 membrane lots). Shaded volumes were used for the calculation of protein recovery in Table 5.

Table 3. Typical Concentrate Volume vs. Spin Time (35° Fixed-angle rotor)

	Concentrate volume (µL)				
Spin time (min)	3K device	10K device	30K device	50K device	100K device
10	613	97	42	23	53
15	–	54	30	–	30
20	170	35	22	15	26
25	118	–	–	–	–
30	92	–	–	–	–
40	62	–	–	–	–

Spin conditions: 7,500 × g for 3K 10K, 30K, and 50K, 5,000 × g for 100K, room temperature, 4 mL starting volume. Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=6 (mean value of 3 membrane lots). Shaded volumes were used for the calculation of protein recovery in Table 5.

Protein Retention and Concentrate Recovery

The membranes used in Amicon® Ultra devices are characterized by a molecular weight cutoff (MWCO); that is, their ability 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. For best results, use a membrane with a MWCO at least two times smaller than the molecular weight of the protein solute that one intends to concentrate. Refer to Table 4.

Table 4. Typical Retention of Protein Markers

Marker/Concentration	Molecular Weight	Device MWCO	% Retention Swinging-bucket	% Retention Fixed-angle	Spin Time (min)
α-Chymotrypsinogen (1 mg/mL)	25,000	3K	>95	>95	40
Cytochrome c (0.25 mg/mL)	12,400		>95	>95	40
Vitamin B-12 (0.2 mg/mL)	1,350		<35	<35	40
α-Chymotrypsinogen (1 mg/mL)	25,000	10K	>95	>95	15
Cytochrome c (0.25 mg/mL)	12,400		>95	>95	15
Vitamin B-12 (0.2 mg/mL)	1,350		<15	<15	15
BSA (1 mg/mL)	67,000	30K	>95	>95	10
Ovalbumin (1 mg/mL)	45,000		>90	>90	10
Cytochrome c (0.25 mg/mL)	12,400		<20	<20	10
Vitamin B-12 (0.2 mg/mL)	1,350		<10	<10	10
BSA (1 mg/mL)	67,000	50K	>95	>95	10 (SB), 5 (FA)
Ovalbumin (1 mg/mL)	45,000		~60	~65	10 (SB), 5 (FA)
Cytochrome c (0.25 mg/mL)	12,400		<10	<10	10 (SB), 5 (FA)
Thyroglobulin (0.5 mg/mL)	677,000	100K	>95	>95	15
IgG (1 mg/mL)	156,000		>90	>90	15
Ovalbumin (1 mg/mL)	45,000		<25	<20	15

Spin Conditions: Swinging-bucket (SB) rotor, 4,000 × g, **or** 35° fixed-angle (FA) rotor, 7,500 × g for 3K, 10K, 30K, and 50K, 5,000 × g for 100K, 4 mL starting volume, room temperature, n=6 (mean value of 3 membrane lots).

Protein Retention and Concentrate Recovery, continued

Factors that determine sample recovery include the nature of the protein solute relative to the device MWCO chosen, starting concentration, and concentration factor. Table 5 provides typical recoveries for Amicon® Ultra-4 devices.

Table 5. Typical Concentrate Recovery

			Concentrate Volume (µL)		Concentration Factor (X)		Concentrate Recovery (%)	
Marker/ Concentration	Device MWCO	Spin Time (min)	Swinging-bucket	Fixed-angle	Swinging-bucket	Fixed-angle	Swinging-bucket	Fixed-angle
Cytochrome c (0.25 mg/mL)	3K	40	94	62	43.5	65.0	98.2	96.7
Cytochrome c (0.25 mg/mL)	10K	15	76	54	52.3	76.6	97.3	98.5
BSA (1 mg/mL)	30K	10	73	42	56.1	98.6	95.8	95.0
BSA (1 mg/mL)	50K	10	32	23	137.0	177.4	98.8	92.8
IgG (1 mg/mL)	100K	15 (SB), 10 (FA)	36	53	115.9	56.8	92.2	91.3

Spin Conditions: Swinging-bucket (SB) rotor, 4,000 × g, **or** 35° fixed-angle (FA) rotor, 7,500 × g for 3K, 10K, 30K, and 50K, 5,000 × g for 100K, 4 mL starting volume, room temperature, n=6 (mean value of 3 membrane lots). The shaded volumes were taken from Tables 2 and 3.

Maximizing Sample Recovery

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

- Adsorptive losses depend upon solute 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 the 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 Amicon® Ultra-4 device.

How to Quantify Recoveries

Calculate total recovery, percent concentrate recovery, and percent filtrate recovery using the method below. The procedure 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 Procedure

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. Before use, separately weigh the empty filter device, the centrifuge tube, and an empty tube for concentrate collection.
2. Fill filter device with solution and reweigh.
3. Assemble device and centrifuge per instructions.
4. Collect the concentrate with a pipettor and dispense it into the preweighed concentrate collection tube.
5. Remove the device from the centrifuge tube and weigh the centrifuge tube and concentrate collection tube.
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	
Swinging-bucket and fixed-angle rotors (45° and 35°)	4.0 mL
Fixed-angle rotor (23°)	3.5 mL
Typical final concentrate volume	50–100 µL
Maximum relative centrifugal force	
Swinging-bucket rotor	4,000 × g
Fixed-angle rotor	7,500 × g for 3K, 10K, 30K, and 50K MWCO 5,000 x g for 100K MWCO
Active membrane area	3 cm^2
Dimensions	
Filter device in tube (capped)	
Length: 124 mm (4.9 in.)	Diameter: 17.3 mm (0.7 in.)
Filter device	
Length: 73.4 mm (2.9 in.)	Diameter: 17.2 mm (0.7 in.)
Materials of Construction	
Filter device	Copolymer styrene/butadiene
Membrane	Ultracel® low binding regenerated cellulose
Filtrate tube	Polypropylene
Filtrate cap and liner	Polyethylene

Chemical Compatibility

Amicon® Ultra centrifugal devices are intended for use with biological fluids and aqueous solutions. Before use, check the sample for chemical compatibility with the device.

Table 6. Chemical Compatibility of Amicon® Ultra Filter Devices

Acids	Concentration		Concentration
Acetic acid	≤ 50%*	Phosphoric acid	≤ 30%
Formic acid	≤ 5%*	Sulfamic acid	≤ 3%
Hydrochloric acid	≤ 1.0 M	Sulfuric acid	≤ 3%
Lactic acid	≤ 50%	Trichloroacetic acid (TCA)	≤ 10%*
Nitric acid	≤ 10%	Trifluoroacetic acid (TFA)	≤ 30%*
Alkalis			
Ammonium hydroxide	≤ 10%	Sodium hydroxide	≤ 0.5 M
Alcohols			
n-Butanol	≤ 70%	Isopropanol	≤ 70%
Ethanol	≤ 70%	Methanol	≤ 60%
Detergents			
Alconox® detergent	≤ 1%	Sodium dodecyl sulfate (SDS)	≤ 0.1%
CHAPS detergent	≤ 0.1%	Tergazyme™ detergent	≤ 1%
Lubrol® PX detergent	≤ 0.1%	Triton® X-100 surfactant	≤ 0.1%
Nonidet® P-40 surfactant	≤ 2%	Tween® 20 surfactant	≤ 0.1%
Sodium deoxycholate	≤ 5%		
Organic solvents			
Acetone	not recommended	Ethyl acetate	not recommended
Acetonitrile	≤ 20%	Formaldehyde	≤ 5%
Benzene	not recommended	Pyridine	not recommended
Carbon tetrachloride	not recommended	Tetrahydrofuran	not recommended
Chloroform	not recommended	Toluene	not recommended
Dimethyl sulfoxide (DMSO)	≤ 5%*		
Miscellaneous			
Ammonium sulfate	Saturated	Phenol	≤ 1%
Diethyl pyrocarbonate	≤ 0.2%	Phosphate buffer (pH 8.2)	≤ 1 M
Dithiothreitol (DTT)	≤ 0.1 M	Polyethylene glycol	≤ 10%
Glycerine	≤ 70%	Sodium carbonate	≤ 20%
Guanidine HCl	≤ 6 M	Tris buffer (pH 8.2)	≤ 1 M
Imidazole	≤ 100 mM	Urea	≤ 8 M
Mercaptoethanol	≤ 0.1 M		

* Contact with this chemical may cause materials to leach out of the component parts. Solvent blanks are recommended to determine whether leachables represent potential assay interferences.

Product Ordering Information

This section lists the catalogue numbers for Amicon® Ultra Centrifugal Filter Devices. See the Technical Assistance section for contact information. You can purchase these products on-line at www.millipore.com/products.

Initial volume (mL)	Final concentrate volume (µL)	Product	Qty/pk	3K	10K	30K	50K	100K
0.5	15–20	Amicon® Ultra-0.5 device	8 24 96 500	UFC500308 UFC500324 UFC500396 UFC5003BK	UFC501008 UFC501024 UFC501096 UFC5010BK	UFC503008 UFC503024 UFC503096 UFC5030BK	UFC505008 UFC505024 UFC505096 UFC5050BK	UFC510008 UFC510024 UFC510096 UFC5100BK
Amicon® Ultra-0.5 Collection Tubes			96	UFC50VL96				
2	30–70	Amicon® Ultra-2 device	24	UFC200324	UFC201024	UFC203024	UFC205024	UFC210024
4	50–100	Amicon® Ultra-4 device	8 24 96	UFC800308 UFC800324 UFC800396	UFC801008* UFC801024* UFC801096*	UFC803008 UFC803024 UFC803096	UFC805008 UFC805024 UFC805096	UFC810008 UFC810024 UFC810096
15	150–300	Amicon® Ultra-15 device	8 24 96	UFC900308 UFC900324 UFC900396	UFC901008* UFC901024* UFC901096*	UFC903008 UFC903024 UFC903096	UFC905008 UFC905024 UFC905096	UFC910008 UFC910024 UFC910096

* Amicon® Ultra-4 and -15 10K devices are for in vitro diagnostic use. All other devices are for research use only.

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").

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