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User Guide

Amicon® Ultra-15 Centrifugal Filter Devices

for volumes up to 15 mL

Amicon® Ultra-15 3K, 30K, 50K, and 100K devices for research use only; not for use in diagnostic procedures

Introduction

Amicon® Ultra-15 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 15 to 60 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 or fixed-angle rotor. Amicon® Ultra-15 devices are supplied non-sterile and for single use only.

The Amicon® Ultra-15 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
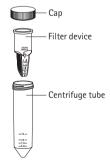- Amicon® Ultra 100K device – 100,000 MWCO

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-15 device is supplied with a cap, a filter device, and a centrifuge tube.

- Cap
- Filter device
- Centrifuge tube

Required Equipment

- Centrifuge with swinging-bucket or fixed-angle rotor with wells/carriers that can accommodate 50 mL 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.

Shelf Life

Shelf life is 3 years from date of manufacture.

Rinsing Before Use

The ultrafiltration membranes in Amicon® Ultra-15 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-15 Centrifugal Filter Devices

1. Add up to 15 mL of sample (12 mL if using a fixed-angle rotor) to the Amicon® Ultra filter device.
2. Place capped filter device into centrifuge rotor; counterbalance with a similar device.
3. **When using a swinging-bucket rotor, s**pin the device at 4,000 × g maximum for approximately 15–60 minutes.

   When using a fixed-angle rotor, orient the device with the membrane panel facing up and spin at 5,000 × g maximum for approximately 15–60 minutes.
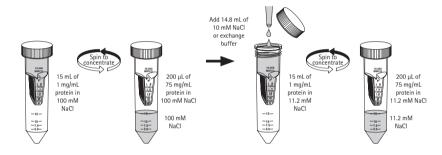
   NOTE: Refer to Figures 1 and 2, and Tables 1 and 2 for typical spin times.

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-15 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.
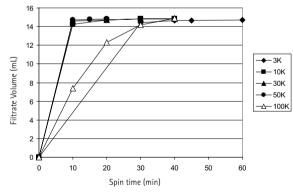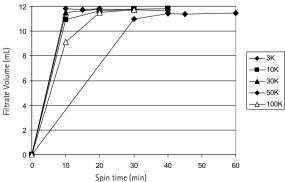

Performance

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 15 mL sample is approximately 15 to 60 minutes (depending on device molecular weight cutoff)). While most of the sample is filtered in the first 15 to 30 minutes of centrifugation, the lowest concentrate volume (150–300 µL) is reached after spinning for 15 to 60 minutes.

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

Spin conditions: 4,000 × g, room temperature, 15 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: 5,000 × g, room temperature, 12 mL starting volume.
Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=6.

Flow Rate, continued

Table 1. Typical Concentrate Volume vs. Spin Time (Swinging-bucket rotor)

Spin time (min)	Concentrate volume (µL)				
	3K device	10K device	30K device	50K device	100K device
10	–	668	361	249	7,420
15	–	–	–	201	–
20	–	219	206	175	2,216
30	537	145	155	–	244
40	331	146	135	–	141
45	299	–	–	–	–
60	209	–	–	–	–

Spin conditions: 4,000 × g, room temperature, 15 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 4.

Table 2. Typical Concentrate Volume vs. Spin Time (Fixed-angle rotor)

Spin time (min)	Concentrate volume (µL)				
	3K device	10K device	30K device	50K device	100K device
10	–	994	411	136	2,760
15	–	–	–	113	–
20	–	273	140	91	400
30	947	159	111	–	166
40	529	101	90	–	–
45	462	–	–	–	–
60	268	–	–	–	–

Spin conditions: 5,000 × g, room temperature, 12 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 4.

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 3.

Table 3. 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	60
Cytochrome c (0.25 mg/mL)	12,400		>95	>95	60
Vitamin B-12 (0.2 mg/mL)	1,350		<25	<25	60
α-Chymotrypsinogen (1 mg/mL)	25,000	10K	>95	>95	30
Cytochrome c (0.25 mg/mL)	12,400		>95	>95	30
Vitamin B-12 (0.2 mg/mL)	1,350		<5	<5	30
BSA (1 mg/mL)	67,000	30K	>95	>95	20
Ovalbumin (1 mg/mL)	45,000		>95	>95	20
Cytochrome c (0.25 mg/mL)	12,400		<10	<10	20
Vitamin B-12 (0.2 mg/mL)	1,350		<5	<5	20
BSA (1 mg/mL)	67,000	50K	>90	>90	10
Ovalbumin (1 mg/mL)	45,000		~65	~55	10
Cytochrome c (0.25 mg/mL)	12,400		<5	<5	10
Thyroglobulin (0.5 mg/mL)	677,000	100K	>90	>90	20
IgG (1 mg/mL)	156,000		>90	>90	20
Ovalbumin (1 mg/mL)	45,000		<25	<15	20

Spin Conditions: Swinging-bucket rotor (4,000 × g, 15 mL starting volume), or fixed-angle rotor, (5,000 × g, 12 mL starting volume), room temperature, n=6 (mean value of 3 membrane lots).

Factors that determine sample recovery include the nature of the protein solute relative to the device MWCO chosen, starting concentration, and concentration factor. Table 4 provides typical recoveries for Amicon® Ultra-15 devices.

Table 4. Typical Concentrate Recovery

Marker/Concentration	Device MWCO	Spin Time (min)	Concentrate Volume (µL)		Concentration Factor (x)		Concentrate Recovery (%)	
			Swinging–bucket	Fixed-angle	Swinging–bucket	Fixed-angle	Swinging–bucket	Fixed-angle
Cytochrome c (0.25 mg/mL)	3K	60	209	268	73.8	44.6	93.8	94.4
Cytochrome c (0.25 mg/mL)	10K	20	219	273	71.4	44.8	95.9	95.1
BSA (1 mg/mL)	30K	20	206	140	72.8	85.5	96.2	95.5
BSA (1 mg/mL)	50K	15	201	113	77.7	106.8	90.7	92.0
IgG (1 mg/mL)	100K	30	244	166	67.6	71.9	81.0	82.9

Spin Conditions: Swinging-bucket rotor (4,000 × g, 15 mL starting volume), or fixed-angle rotor, (5,000 × g, 12 mL starting volume), room temperature, n=6 (mean value of 3 membrane lots). The shaded volumes were taken from Tables 1 and 2.

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 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-15 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	15.0 mL
Fixed-angle rotor	12.0 mL

Typical final concentrate volume   150–300 µL

Maximum relative centrifugal force

Swinging-bucket rotor	4,000 × g
Fixed-angle rotor	5,000 × g

Active membrane area   7.6 cm²

Dimensions

Filter device in tube (capped)

    Length: 119 mm (4.7 in.)   Diameter: 33.5 mm (1.3 in.)

Filter device

    Length: 72.0 mm (2.8 in.)   Diameter: 29.7 mm (1.2 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 5. 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 Ultrafiltration 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	UFC500308	UFC501008	UFC503008	UFC505008	UFC510008
			24	UFC500324	UFC501024	UFC503024	UFC505024	UFC510024
			96	UFC500396	UFC501096	UFC503096	UFC505096	UFC510096
			500	UFC5003BK	UFC5010BK	UFC5030BK	UFC5050BK	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	UFC800308	UFC801008*	UFC803008	UFC805008	UFC810008
			24	UFC800324	UFC801024*	UFC803024	UFC805024	UFC810024
			96	UFC800396	UFC801096*	UFC803096	UFC805096	UFC810096
15	150–300	Amicon® Ultra-15 device	8	UFC900308	UFC901008*	UFC903008	UFC905008	UFC910008
			24	UFC900324	UFC901024*	UFC903024	UFC905024	UFC910024
			96	UFC900396	UFC901096*	UFC903096	UFC905096	UFC910096

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

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Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms (within the "Terms and Conditions of Sale" applicable to your purchase transaction).