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Amicon ultra-0.5 Filter Devices User Manual

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Introduction

Millipore's Amicon® Ultra-0.5 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 30-fold concentration. Typical processing time is 10 to 30 minutes depending on the Nominal Molecular Weight Limit
(NMWL). 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. Efficient recovery of the concentrated sample (retained species) is achieved by a convenient reverse spin step after collecting the filtrate. Amicon Ultra-0.5 devices are supplied non-sterile and are for single use only.

The Amicon Ultra-0.5 product line includes 5 different cutoffs (Nominal Molecular Weight Limit, NMWL). These devices are for research use only and not for use in diagnostic procedures.

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

Applications

- The 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
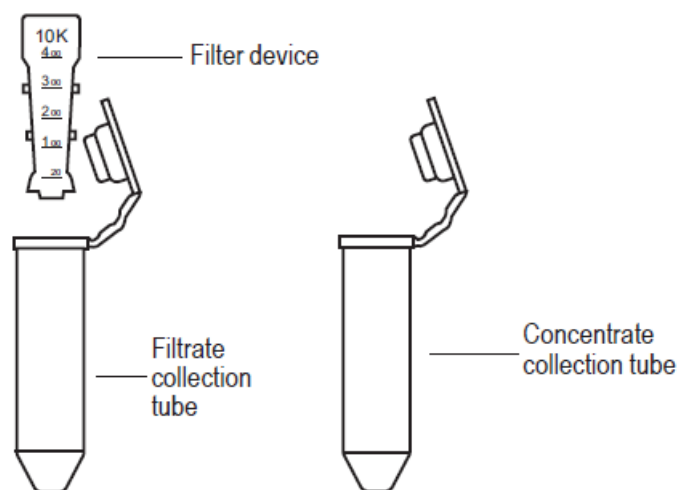
The Amicon Ultra-0.5 device is supplied with two microcentrifuge tubes. During operation, one tube is used to collect filtrate; the other to recover the concentrated sample.

Required Equipment

- Centrifuge with fixed angle rotor that can accommodate 1.5 mL microcentrifuge tubes CAUTION: To avoid damage to the device during centrifugation, check clearance before spinning.

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

Device Storage
Store at room temperature.

Specifications

- Maximum initial sample volume 500 µL
- Typical final concentrate volume 15–20 µL
- Recommended relative centrifugal force 14,000 × g for concentration spin 1,000 × g for reverse spin
- Maximum relative centrifugal force 15,000 × g
- Active membrane area 1 cm2
- Hold-up volume < 5 µL
  ### Dimensions
- Length (concentration mode; device in tube): 49.9 mm (1.96 in.)
- Length (reverse spin; device upside down in tube): 47.4 mm (1.87 in.)
- Tube (cap closed) Diameter: 10.8 mm (0.43 in.) Length: 42.1 mm (1.66 in.)
- Filter device Diameter: 9.4 mm (0.37 in.) Length: 29.5 mm (1.16 in.)
  ### Materials of Construction
- Filter devise Copolymer styrene/butadiene
- Membrane Millipore Ultracel® low binding regenerated cellulose
- Collection tube Polypropylene

The ultrafiltration membranes in Amicon Ultra-0.5 devices contain trace amounts of the following:

- Amicon Ultra 3K devices: glycerine
- Amicon Ultra 10K device: trimethylene glycol (TEG)
- Amicon Ultra 30K devices: glycerine
- Amicon Ultra 50K devices: glycerine
- Amicon Ultra 100K devices: glycerine

If these materials interfere with the analysis, pre-rinse the device with buffer or Milli-Q® water. If the 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 pre-rinsing, leave the fluid on the membrane until the device is used.

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 1. Chemical Compatibility of Amicon Ultra Filter Devices

Acids

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%	Lubrol® PX detergent	≤ 0.1%
CHAPS detergent	≤ 0.1%	Nonidet™ P-40 surfactant	≤ 2%

Sodium deoxycholate	≤ 5%	Triton® X-100 surfactant	≤ 0.1%
Sodium dodecyl sulfate (SDS)	≤ 0.1%	Tween® 20 surfactant	≤ 0.1%
Terg-A-Zyme® detergent	≤ 1%		

* 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.

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.

How to Use Amicon Ultra-0.5 Centrifugal Filter Devices

1. Insert the Amicon Ultra-0.5 device into one of the provided microcentrifuge tubes.
2. Add up to 500 µL of sample to the Amicon Ultra filter device and cap it.
3. Place capped filter device into the centrifuge rotor, aligning the cap strap toward the center of the rotor; counterbalance with a similar device.
4. Spin the device at 14,000 × g for approximately 10–30 minutes depending on the NMWL of the device used. Refer to Figure 1 and table 3 for typical spin times.

Add sample   Cap   Spin at 14,000 × g
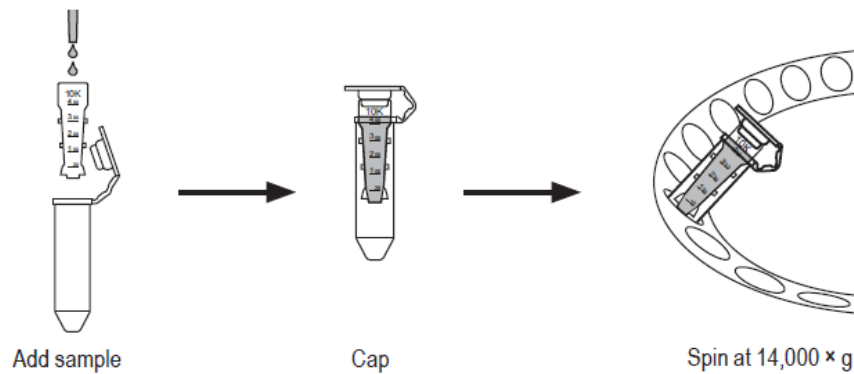
5. Remove the assembled device from the centrifuge and separate the Amicon Ultra filter device from the microcentrifuge tube.

6. To recover the concentrated solute, place the Amicon Ultra filter device upside down in a clean microcentrifuge tube. Place in a centrifuge, aligning the open cap towards the center of the rotor; counterbalance with a similar device. Spin for 2 minutes at 1,000 × g to transfer the concentrated sample from the device to the tube. The ultrafiltrate can be stored in the centrifuge tube.

NOTE: For optimal recovery, perform the reverse spin immediately.

How to Quantitate Recoveries

Calculate total recovery, percent concentrate, and percent filtrate 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, 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 recoveries can be quantitated 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 a solution and reweigh.

3. Assemble the device in filtrate collection tube and centrifuge per instructions.

4. Collect the concentrate by a reverse spin into the pre-weighed concentrate tube.

5. Remove the device from the concentrate collection tube and weigh the filtrate and concentrate collection tubes.

6. Subtract weight of empty devices/tubes to calculate the weights of starting material, filtrate, and concentrate.

7. Assay the filtrate and concentrate to determine solute concentration.

8. Calculate recoveries using the weight/volume data and the measured concentrations as follows:

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

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

% total recovery = % concentrate recovery + % 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

Co = original starting material concentration Cf = filtrate concentration

Performance – DNA Concentration

The Amicon Ultra-0.5 30K device provides the best balance between recovery and spin time for double-stranded DNA for base pairs ranging from 137 to 1159. To achieve maximum PCR product recovery and primer removal with primers greater than 20 bases, one or two additional spins with Tris-EDTA
(TE) buffer are recommended.

Table 2. Typical recovery of nucleotides from Amicon Ultra-0.5 30K device

PCR Product (base pairs)	PCR Primer (bases)	PCR Recovery (%)	PCR Primer Removal (%)	TE Washes (number)
137	10	≥ 95	≥ 90	0
	20	≥ 90	≥ 85	1
	48	≥ 90	≥ 75	2
301	10	≥ 90	≥ 90	0
	20	≥ 85	≥ 90	1
	48	≥ 90	≥ 80	2
648	10	≥ 95	≥ 90	0
	20	≥ 90	≥ 90	1
	48	≥ 95	≥ 90	2
1159	10	≥ 90	≥ 90	0
	20	≥ 90	≥ 95	1
	48	≥ 95	≥ 95	2

Spin conditions: 40° fixed angle rotor, 14,000 × g, room temperature, 500 µL starting volume, 20–30 µL final volume, 10-minute spin, n=12.

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. Figure 1 and Table 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 500 µL sample in a fixed angle rotor is approximately 10–30 minutes (depending on device's nominal molecular weight limit). While most of the sample is filtered in the first 5 to 10 minutes of centrifugation, the lowest concentrate volume (15–20 µL) is reached after spinning for 10–30 minutes.

Figure 1. Typical Filtrate Volume vs. Spin Time
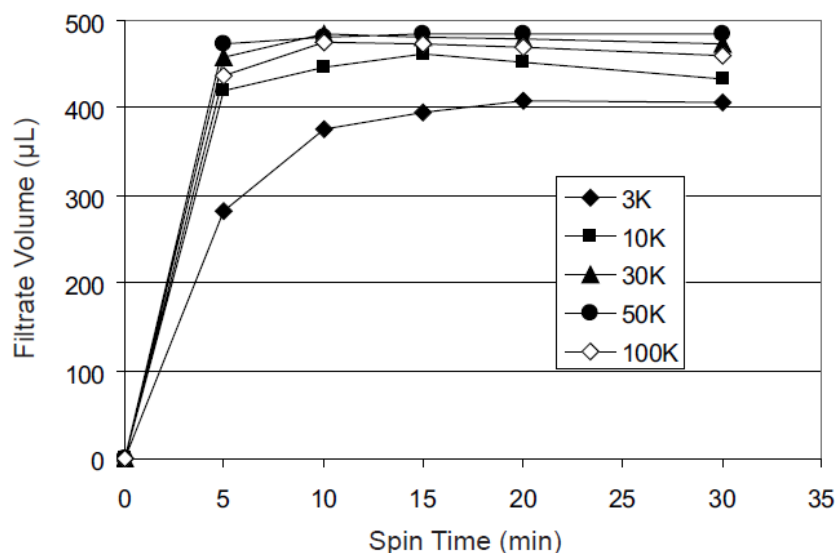
Spin conditions: 40° fixed angle rotor, 14,000 × g, room temperature, 500 µL starting volume. Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=8.

Table 3. Typical Concentrate Volume / Concentration Factor vs. Spin Time

Concentrate volume / Concentration Factor

3K device   10K device   30K device   50K device   100K devices

Spin time (min)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)	Conc. Volume (µL)	Conc. Factor (x)
5	215	2	66	7	42	12	28	18	58	9
10	114	4	35	14	23	22	20	25	19	26
15	80	6	22	22	19	27	17	30	15	33
20	62	8	20	24	17	30	15	33	13	36
30	48	10	15	31	15	32	15	36	11	41

Spin conditions: 40° fixed angle rotor, 14,000 × g, room temperature, 500 µL starting volume. Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=12. 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 nominal molecular weight limit (NMWL); that is, their ability to retain molecules above a specified molecular weight. Solutes with molecular weights close to the NMWL 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. Millipore recommends using a membrane with an NMWL at least two times smaller than the molecular weight of the protein solute that one intends to concentrate. Refer to Table 4 for "Typical Retention of Protein Markers".

Table 4: Typical Retention of Protein Markers

Marker/Concentration	Weight	NMWL	% Retention	(min)
α-Chymotrypsinogen (1 mg/mL)	25,000	3K	> 95	30
Cytochrome c (0.25 mg/mL)	12,400		> 95	30
Vitamin B-12 (0.2 mg/mL)	1,350		< 42	30
α-Chymotrypsinogen (1 mg/mL)	25,000	10K	> 95	15
Cytochrome c (0.25 mg/mL)	12,400		> 95	15
Vitamin B-12 (0.2 mg/mL)	1,350		< 42	15
BSA (1 mg/mL)	67,000	30K	> 95	10
Ovalbumin (1 mg/mL)	45,000		> 95	10
Cytochrome c (0.25 mg/mL)	12,400		< 35	10
BSA (1 mg/mL)	67,000	50K	> 95	10
Ovalbumin (1 mg/mL)	45,000		~ 40	10
Cytochrome c (0.25 mg/mL)	12,400		< 20	10
Thyroglobulin (0.5 mg/mL)	677,000	100K	> 95	10
IgG (1 mg/mL)	156,000		> 95	10
Ovalbumin (1 mg/mL)	45,000		< 30	10

Factors that determine sample recovery include the nature of the protein solute relative to the device NMWL chose, starting concentration, and concentration factor. Table 5 provides typical recoveries for Amicon Ultra-0.5 devices.

Table 5. Typical Concentrate Recovery

Marker/ Concentration	Molecular Weight	Device NMWL	Spin Time (min)	Concentrate Volume (µL)	Concentration Factor (x)	Concentrate Recovery (%)
Cytochrome c (0.25 mg/mL)	12,400	3K	30	48	10	98
Cytochrome c (0.25 mg/mL)	12,400	10K	15	22	22	93
BSA (1 mg/mL)	67,000	30K	10	23	22	97
BSA (1 mg/mL)	67,000	50K	10	20	25	92
IgG (1 mg/mL)	156,000	100K	10	19	26	92

Spin Conditions: 40° fixed angle rotor, 14,000 × g, room temperature, 500 µL starting volume, n=12. The shaded volumes were taken from Table 3.

Maximizing Sample Recovery

Low sample recovery in the concentrate may be due to adsorptive losses, over-concentration, or passage of the 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 NMWL Amicon Ultra-0.5 device.

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-0.5 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 micro solute has been sufficiently reduced. See the example below.
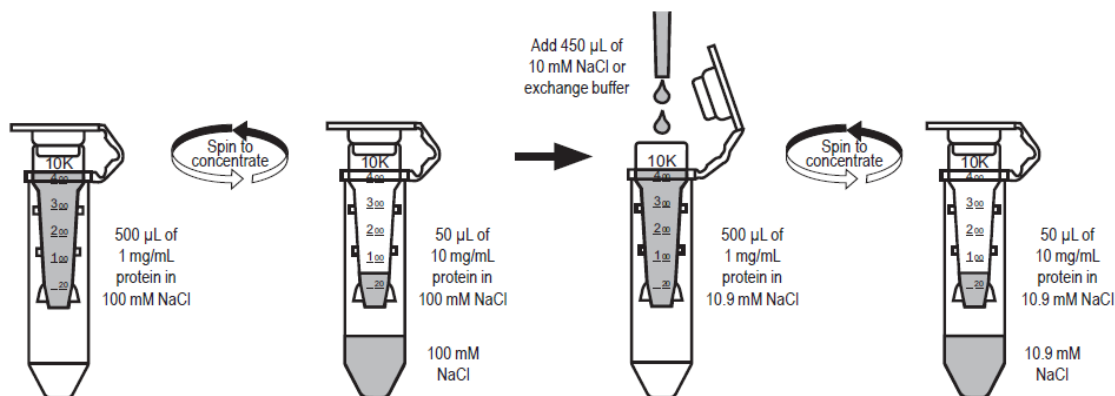
Centrifugal Product Ordering Information

Initial volume (mL)	Final concentrate volume (µL)	Product	Qty / pk	3,000 NMWL	10,000 NMWL	30,000 NMWL	50,000 NMWL	100,000 NMWL
		Amicon	8	UFC500308	UFC501008	UFC503008	UFC505008	UFC5100008
0.5	20	Ultra-0.5 device	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				
		Amicon	8	UFC800308	UFC801008*	UFC803008	UFC805008	UFC810008
4	30–70	Ultra-4	24	UFC800324	UFC801024*	UFC803024	UFC805024	UFC810024
		device	96	UFC800396	UFC801096*	UFC803096	UFC805096	UFC810096
		Amicon	8	UFC900308	UFC901008*	UFC903008	UFC905008	UFC910008
15	150–300	Ultra-15	24	UFC900324	UFC901024*	UFC903024	UFC905024	UFC910024
		device	96	UFC900396	UFC901096*	UFC903096	UFC905096	UFC910096

Related Products

Initial volume (mL)	Final concentrate volume (µL)	Product	Qty / pk	3,000 NMWL	10,000 NMWL	30,000 NMWL	50,000 NMWL	100,000 NMWL
70	350	Centricon® Plus-70 device	8	UFC700508	UFC701008	UFC703008	N/A	UFC710008
0.5	20	MultiScreen® 96 well plate/ Ultracel-10 membrane	10	N/A	MAUF01010	N/A	N/A	N/A

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 catalog for the phone number of the office nearest you or go to our website 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**.

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