User Guide

Amicon® Ultra-0.5 Centrifugal Filter Devices

for volumes up to 500 µL

For research use only;
not for use in diagnostic procedures.

Introduction

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 Molecular Weight Cut off (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. 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.

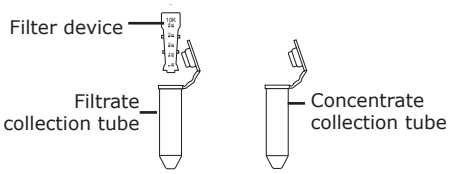
The Amicon® Ultra-0.5 product line includes 5 different cutoffs (Molecular Weight Cut Off, MWCO). These devices are for research use only and not for use in diagnostic procedures.

- 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

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-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 intended use. See the "How to Quantify Recoveries" section.

Device Storage

Store at room temperature.

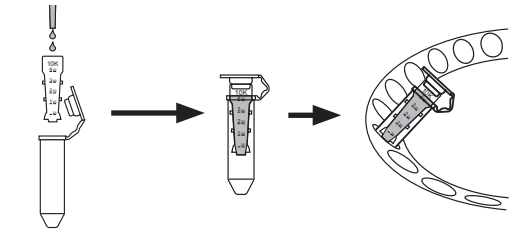
Prerinsing

The ultrafiltration membranes in Amicon® Ultra-0.5 devices contain trace amounts of glycerine. If this material interferes with analysis, pre-rinse the device with buffer or Milli-Q® water. 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 pre-rinsing, leave fluid on the membrane until the device is used.

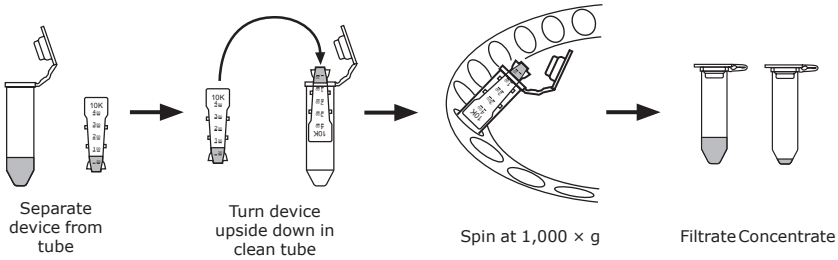
How to Use Amicon® Ultra-0.5 Centrifugal Filter Devices

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



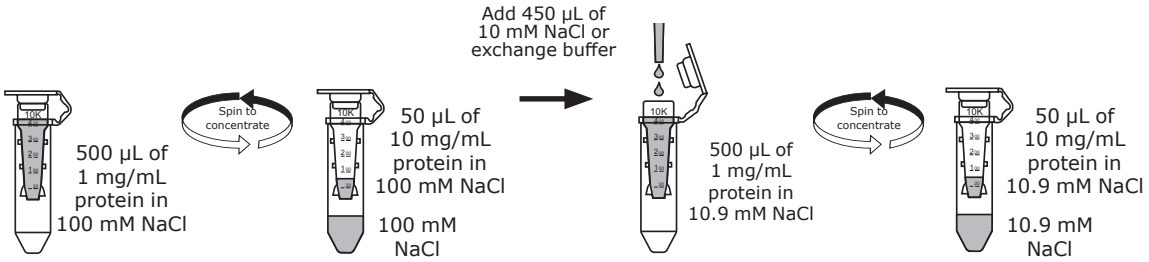
6. Remove the assembled device from the centrifuge and separate the Amicon® Ultra filter device from the microcentrifuge tube.
7. To recover the concentrated solute, place the Amicon® Ultra filter device upside down in a clean microcentrifuge tube. Place in centrifuge, aligning open cap towards the center 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.



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, 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. See example below.



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 1,159. 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 1. Typical recovery of nucleotides from Amicon® Ultra-0.5 devices

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
657	10	≥ 95	≥ 90	0
	20	≥ 90	≥ 90	1
	48	≥ 95	≥ 90	2
1,159	10	≥ 90	≥ 90	0
	20	≥ 90	≥ 95	1
	48	≥ 95	≥ 95	2

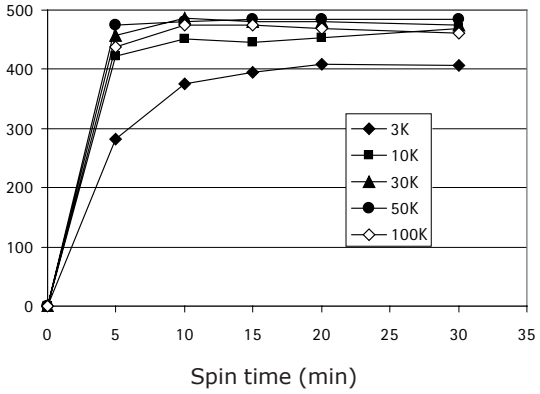
Spin conditions: 40° fixed angle rotor, 14,000 × g, room temperature, 100 µL PCR and 400 µL TE buffer for a starting volume of 500 µL, 20–30 µL final volume, 10 minute spin, n=12.

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. Figure 1 and Table 2 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 is approximately 10 to 30 minutes (depending on device 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 to 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 2. Typical Concentrate Volume / Concentration Factor vs. Spin Time

	3K device		10K device		30K device		50K device		100K device	
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	74	7	42	12	28	18	58	9
10	114	4	42	12	23	22	20	25	19	26
15	80	6	27	18	19	27	17	30	15	33
20	62	8	20	25	17	30	15	33	13	36
30	48	10	17	30	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 molecular weight cut off (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. 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. Refer to Table 3.

Table 3. Typical Retention of Protein Markers.

Marker/Concentration	Molecular Weight	Device MWCO	% Retention	Spin Time (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		<23	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

Spin Conditions: 40° fixed angle rotor, 14,000 x g, room temperature, 500 µL starting volume, n=12.

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-0.5 devices.

Table 4. Typical Concentrate Recovery.

Marker/ Concentration	Molecular Weight	Device MWCO	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	27	18	95
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 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-0.5 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. 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 reverse 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:

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

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

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

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	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 cm^2
Hold-up volume	< 5 µL

Dimensions

Filter device and tube

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 device	Copolymer styrene/butadiene
Membrane	Ultracel® low binding regenerated cellulose
Collection tubes	Polypropylene

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%	Triton® X-100 surfactant	≤ 0.1%
CHAPS detergent	≤ 0.1%	Tween® 20 surfactant	≤ 0.1%
Lubrol® PX detergent	≤ 0.1%		
Nonidet™ P-40 surfactant	≤ 2%		
Sodium deoxycholate	≤ 5%		
Sodium dodecyl sulfate (SDS)	≤ 0.1%		
Tergazyme® detergent	≤ 1%		
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.sigmaaldrich.com/products.

MWCO	Qty/ pk	Amicon® Ultra-0.5 device	Amicon® Ultra-2 device	Amicon® Ultra-4 device	Amicon® Ultra-15 device
3K	8 24 96 500	UFC500308 UFC500324 UFC500396 UFC5003BK	UFC200324	UFC800308 UFC800324 UFC800396	UFC900308 UFC900324 UFC900396
10K	8 24 96 500	UFC501008 UFC501024 UFC501096 UFC5010BK	UFC201024	UFC801008 UFC801024 UFC801096	UFC901008 UFC901024 UFC901096
10K IVD*	8 24 96			UFC801008D UFC801024D UFC801096D	UFC901008D UFC901024D UFC901096D
30K	8 24 96 500	UFC503008 UFC503024 UFC503096 UFC5030BK	UFC203024	UFC803008 UFC803024 UFC803096	UFC903008 UFC903024 UFC903096
50K	8 24 96 500	UFC505008 UFC505024 UFC505096 UFC5050BK	UFC205024	UFC805008 UFC805024 UFC805096	UFC905008 UFC905024 UFC905096
100K	8 24 96 500	UFC510008 UFC510024 UFC510096 UFC5100BK	UFC210024	UFC810008 UFC810024 UFC810096	UFC910008 UFC910024 UFC910096

* Amicon® Ultra-4 and -15 10K devices are for in vitro diagnostic (IVD) 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.

Contact Information

For the location of the office nearest you, go to www.sigmaaldrich.com/offices.

Technical Assistance

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

Standard Warranty

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

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