User Guide

Amicon® Ultra-2 Centrifugal Filter Devices

for volumes up to 2 mL

For research use only;
not for use in diagnostic procedures

Introduction

Amicon® Ultra-2 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 50-fold concentration. Typical processing time is 10 to 60 minutes depending on 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. The device can be spun in a swinging bucket or fixed angle rotor. Amicon® Ultra-2 devices are supplied non-sterile and are for single use only.

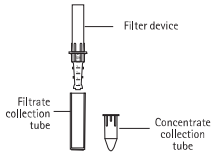
The Amicon® Ultra-2 product line includes 5 different cutoffs (Nominal Molecular Weight Limit, NMWL). These devices are for research use only and not for use in diagnostic procedures.

- 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

- 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-2 device is supplied with a two tubes. During operation, one tube is used to collect filtrate; the other to cap the device during concentration and subsequently to recover the concentrated sample.

Required Equipment

Centrifuge with swinging bucket or fixed angle rotor with wells/carriers that can accommodate 17 mm × 100 mm tubes (same well/carrier size as for Amicon® Ultra-4 devices and the former Centricon® device).

CAUTION: To avoid damage to the device during centrifugation, make sure it is properly assembled and seated at the bottom of the rotor. The rim of the concentrate collection tube should be inside the rotor well. 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

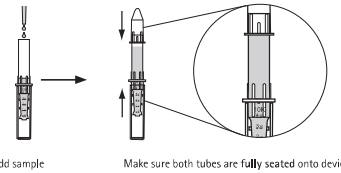
The ultrafiltration membranes in Amicon® Ultra-2 devices contain trace amounts of glycerine. If this material interferes with analysis, pre-rinse the device with buffer or Milli-Q® water. If interference continues, rinse with 0.1 N NaOH followed by a second spin of buffer or Milli-Q® water.

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.

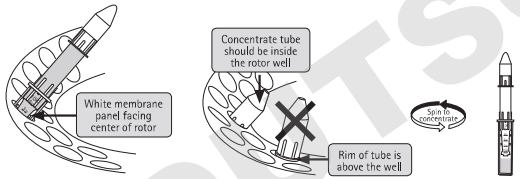
How to Use Amicon® Ultra-2 Centrifugal Filter Devices

1. Insert the Amicon® Ultra-2 device into the filtrate collection tube, making sure that the device is fully seated in the tube.
2. Add up to 2 mL of sample to the device and cover with concentrate collection tube. Push the tube firmly onto the device.

   WARNING: Failure to fully seat the device in the filtrate collection tube and push the concentrate collection tube firmly onto the device may result in the device breaking during centrifugation. See figure below.



3. Place filter device into the centrifuge rotor with one membrane panel facing the center of the rotor (one panel facing up and the other panel facing down). Make sure the device is seated on the bottom of the rotor well and that the rim of the concentrate collection tube is completely inside the well. See figures below. Counterbalance with a similar device.



4. Spin for approximately 10–60 minutes depending on the NMWL of the device used:

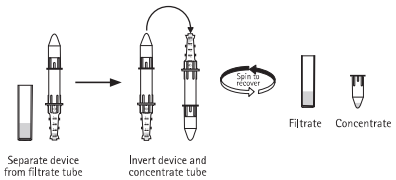
   4,000 × g maximum when using a swinging bucket rotor

   7,500 × g maximum when using a fixed angle rotor

   NOTE: When spinning viscous solutions such as undiluted serum or plasma, do not exceed 5,400 x g.

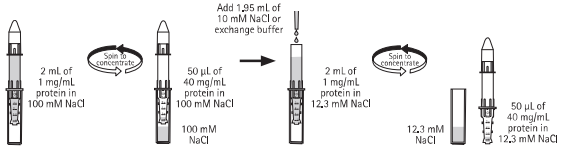
   Refer to Figures 1 and 2, and Tables 2 and 3 for typical spin times.
5. Remove the assembled device from the centrifuge and separate the Amicon® Ultra filter device from the filtrate collection tube.
6. To recover the concentrated solute, invert the Amicon® Ultra filter device and concentrate collection tube. Place in centrifuge and counterbalance with a similar device. Spin for 2 minutes at 1,000 × g to transfer the concentrated sample from the device to the 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-2 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-2 30K device provides the best balance between PCR recovery and PCR primer removal for double-stranded DNA for base pairs ranging from 137 to 1159.

Table 1. Typical Recovery of Nucleotides from the Amicon® Ultra-2 30K Device

		Swinging Bucket Rotor 4,000 × g for 40 min			35° Fixed Angle Rotor 7,500 × g, for 15 min		
PCR Product (base pairs)	PCR Primer (bases)	PCR Recovery (%)	PCR Primer Removal (%)	Final Volume (µL)	PCR Recovery (%)	PCR Primer Removal (%)	Final Volume (µL)
137	10	83	92	44	78	93	27
	20	87	80	43	75	86	22
	48	86	61	41	78	67	25
1159	10	96	98	35	95	98	26
	20	97	93	39	93	93	26
	48	97	82	37	95	82	27

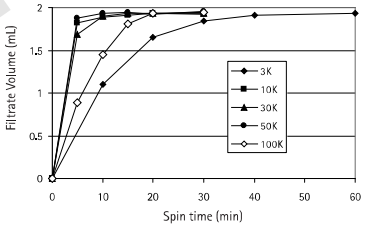
100 µL PCR diluted to 2,000 µL starting volume, n=6

Performance – Protein Concentration

Flow Rate

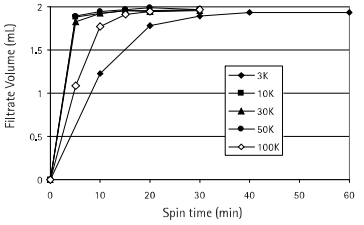
Factors affecting flow rate include sample concentration, starting volume, chemical nature of solute, relative centrifugal force, centrifuge rotor angle, membrane type, and temperature. Figures 1 and 2 and Tables 2 and 3 can be used to estimate the time required to achieve a given volume of filtrate or concentrate for a variety of protein markers. A typical spin time for a 2 mL sample in a fixed angle rotor is approximately 10 to 60 minutes (depending on device nominal molecular weight limit). While most of the sample is filtered in the first 10 to 20 minutes of centrifugation, the lowest concentrate volume (30–70 µL) is reached after spinning for 10 to 60 minutes.

Figure 1. Typical Filtrate Volume vs. Spin Time for Amicon® Ultra-2 Device, Swinging Bucket Rotor



Spin conditions: Swinging bucket rotor, 4,000 × g, room temperature, 2 mL starting volume.
Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=8.

Figure 2. Typical Filtrate Volume vs. Spin Time for Amicon® Ultra-2 Device, Fixed Angle Rotor



Spin conditions: 35° fixed angle rotor, 7,500 × g, room temperature, 2 mL 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, Swinging Bucket Rotor

	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					281	7	91	22	1070	2
10	880	2	190	11	71	27	47	42	523	4
15			96	21	52	38	44	47	167	12
20	317	7	65	31	43	46	38	52	65	31
30	147	30	48	42	39	51	38	53	37	53
40	102	20	44	45						
60	55	32								

Spin conditions: Swinging bucket rotor, 4,000 × g, room temperature, 2 mL starting volume.
Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=8.
Shaded volumes were used for the calculation of protein recovery in Table 5.

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Flow Rate, continued

Table 3. Typical Concentrate Volume / Concentration Factor vs. Spin Time, Fixed Angle Rotor

	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					137	15	80	25	879	2
10	731	3	101	21	51	39	30	71	203	10
15			60	33	37	57	22	90	61	34
20	215	10	39	51	24	85	21	99	32	63
30	106	19	25	80	20	101	18	89	17	115
40	70	29	23	87						
60	45	45								

Spin conditions: 35° fixed angle rotor, 7,500 × g, room temperature, 2 mL starting volume.
Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=8.
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 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. Merck Millipore Ltd. recommends using a membrane with a NMWL 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 NMWL	% Retention Swinging Bucket	Spin Time (min)	% Retention Fixed Angle	Spin Time (min)
α-Chymotrypsinogen (1 mg/mL)	25,000	3K	99	60	99	60
Cytochrome c (0.25 mg/mL)	12,400		100		100	
Vitamin B-12 (0.2 mg/mL)	1,350		6		8	
α-Chymotrypsinogen (1 mg/mL)	25,000	10K	99	30	99	20
Cytochrome c (0.25 mg/mL)	12,400		100		100	
Vitamin B-12 (0.2 mg/mL)	1,350		10		9	
BSA (1 mg/mL)	67,000	30K	100	20	100	15
Ovalbumin (1 mg/mL)	45,000		97		97	
Cytochrome c (0.25 mg/mL)	12,400		16		15	
BSA (1 mg/mL)	67,000	50K	97	15	100	10
Ovalbumin (1 mg/mL)	45,000		50		60	
Cytochrome c (0.25 mg/mL)	12,400		9		17	
Thyroglobulin (0.5 mg/mL)	677,000	100K	94	30	94	20
IgG (1 mg/mL)	156,000		95		95	
Ovalbumin (1 mg/mL)	45,000		12		13	

Spin Conditions: Swinging bucket rotor, 4,000 × g, or 35° fixed angle rotor, 7,500 × g, 2 mL starting volume, room temperature, n=12

Factors that determine sample recovery include the nature of the protein solute relative to the device NMWL chosen, starting concentration, and concentration factor. Table 5 provides typical recoveries for Amicon® Ultra-2 devices.

Table 5. Typical Concentrate Recovery

Marker/ Concentration	Device NMWL	Spin Time (min)		Concentrate Volume (μL)		Concentration Factor (x)		Concentrate Recovery (%)	
		Swinging Bucket	Fixed Angle	Swinging Bucket	Fixed Angle	Swinging Bucket	Fixed Angle	Swinging Bucket	Fixed Angle
Cytochrome c (0.25 mg/mL)	3K	60	60	55	45	32	45	97	96
Cytochrome c (0.25 mg/mL)	10K	30	20	48	39	42	51	98	98
BSA (1 mg/mL)	30K	20	15	43	37	46	57	94	94
BSA (1 mg/mL)	50K	15	10	44	30	47	71	93	87
IgG (1 mg/mL)	100K	30	20	37	32	53	63	88	90

Spin Conditions: Swinging bucket rotor, 4,000 × g, or 35° fixed angle rotor, 7,500 × g, 2 mL starting volume, room temperature, n=8

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 NMWL Amicon® Ultra-2 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:

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

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

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

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 2.0 mL
Typical final concentrate volume 30–70 μL depending on NMWL
Maximum relative centrifugal force
Swinging bucket rotor: 4,000 × g for concentration spin, 1,000 × g for recovery spin
Fixed angle rotor: 7,500 × g for concentration spin, 1,000 × g for recovery spin
NOTE: When spinning viscous solutions such as undiluted serum or plasma, do not exceed 5,400 x g.

Active membrane area 1 cm²
Hold-up volume < 5 μL
Dimensions
Filter device and tube
Length (concentration mode; device in filtrate tube): 119.7 mm (4.71 in.)
Length (recovery spin; device upside down in concentrate tube): 95.3 mm (3.75 in.)
Filter device: Diameter: 15.9 mm (0.63 in.) Length: 70.7 mm (2.78 in.)
Filtrate tube: Diameter: 13.8 mm (0.54 in.) Length: 52.9 mm (2.08 in.)
Concentrate tube: Diameter: 13.7 mm (0.54 in.) Length: 34.5 mm (1.36 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 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%

* 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.

Chemical Compatibility, continued

Detergents	Concentration		Concentration
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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PR03362 Rev. A, 03/12

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