

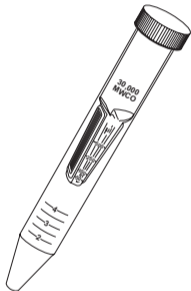
Amicon[®] Ultra-4 Centrifugal Filter Devices

for volumes up to 4 mL

User Guide

Amicon Ultra-4 10K device
for in vitro diagnostic use

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



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Introduction

Millipore's 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 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. 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 non-sterile and are for single use only.**

Introduction, continued

The Amicon Ultra-4 product line includes 5 different cutoffs (Nominal Molecular Weight Limit, NMWL, or Molecular Weight Cutoff, MWCO):

- 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

CE Amicon Ultra-4 10K filter devices are for **in vitro diagnostic use** and can be used to concentrate serum, urine, cerebrospinal fluid, and other body fluids prior to analysis.

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

Applications

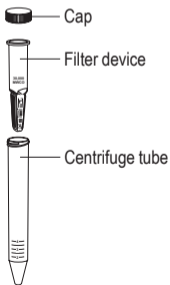
- Concentration of biological samples containing antigens, antibodies, enzymes, nucleic acids (DNA/RNA samples, either single- or double-stranded), microorganisms, column eluates, and purified samples

Applications, continued

- 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 Quantitate Recoveries” section.

Device Storage

Store at room temperature.

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 μ L

Maximum relative centrifugal force

Swinging bucket rotor 4,000 \times g

Fixed angle rotor 7,500 \times g for 3K, 10K, 30K, and 50K NMWL

5,000 \times g for 100K NMWL

Active membrane area 3 cm²

Dimensions

Filter device in tube (capped)

Length: 124 mm (4.88 in.) Diameter: 17.3 mm (0.68 in.)

Filter device

Length: 73.4 mm (2.89 in.) Diameter: 17.2 mm (0.68 in.)

Materials of Construction

Filter device Copolymer styrene/butadiene

Membrane Millipore Ultracel® low binding regenerated cellulose

Filtrate tube Polypropylene

Filtrate cap and liner Polyethylene

Specifications, continued

The ultrafiltration membranes in Amicon Ultra-4 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.

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

* 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, continued	Concentration		Concentration
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%		

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-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 35° fixed angle rotor for Amicon Ultra 3K, 10K, 30K, and 50K devices: Spin at 7,500 × g maximum for approximately 10–40 minutes.

When using a 35° fixed angle rotor 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 3 and 4 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.

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. 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.

Direct Weighing Procedure, continued

4. Collect the concentrate with a pipettor and dispense it into the pre-weighed 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 filtrate and concentrate to determine solute concentration.
8. Calculate recoveries using the weight/volume data and the measured concentrations as follows:

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

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

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

Direct Weighing Procedure, continued

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

Performance - DNA Concentration

Millipore has 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 2. 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 \times g, room temperature, 2 mL starting volume.

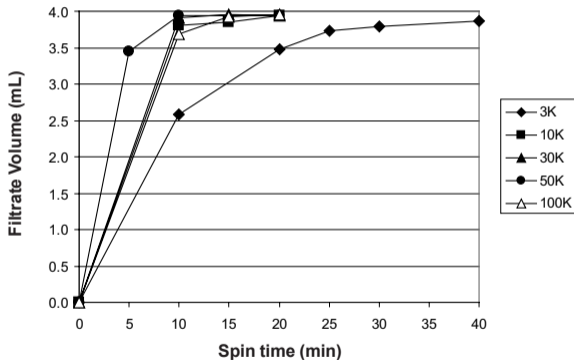
Performance - Protein Concentration

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 3 and 4 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 10–40 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 (30–75 μ L) is reached after spinning for 20–40 minutes.

Flow rate, continued

Figure 1. Typical Filtrate Volume vs. Spin Time for Amicon Ultra-4 Device (Swinging bucket rotor)

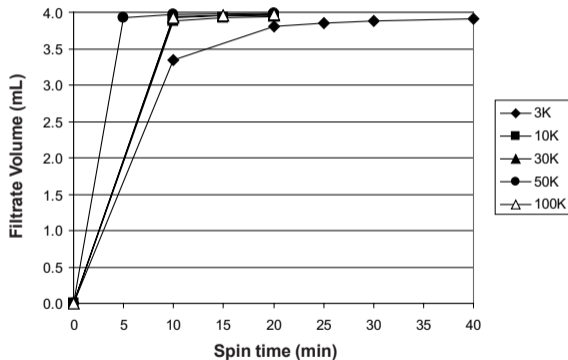


Spin conditions: 4,000 × g, room temperature, 4 mL starting volume.

Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and IgG for 100K, n=6.

Flow rate, continued

Figure 2. Typical Filtrate Volume vs. Spin Time for Amicon Ultra-4 Device (Fixed angle rotor)



Spin conditions: $7,500 \times g$, for 3K, 10K, and 50K, $5,000 \times g$ 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.

Flow rate, continued

Table 3: 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	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 \times 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 6.

Flow rate, continued

Table 4: Typical Concentrate Volume vs. Spin Time (35° Fixed angle rotor)

Spin time (min)	Concentrate volume (μL)				
	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 6.

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 a NMWL at least two times smaller than the molecular weight of the protein solute that one intends to concentrate. Refer to Table 5 for "Typical Retention of Protein Markers".

Protein Retention and Concentrate Recovery, continued

Table 5: Typical Retention of Protein Markers

Marker/Concentration	Molecular Weight	Device NMWL	% 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

Protein Retention and Concentrate Recovery, continued

Factors that determine sample recovery include the nature of the protein solute relative to the device NMWL chosen, starting concentration, and concentration factor. Table 6 provides typical recoveries for Amicon Ultra-4 devices.

Table 6: Typical Concentrate Recovery

Marker/ Concentration	Device NMWL	Spin Time (min)	Concentrate Volume (μ L)		Concentration Factor (x)		Concentrate Recovery (%)	
			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 for tables 5 and 6: Swinging bucket (SB) rotor, 4,000 \times g, or 35° fixed angle (FA) rotor, 7,500 \times g for 3K, 10K, 30K, and 50K, 5,000 \times g for 100K, 4 mL starting volume, room temperature, n=6 (mean value of 3 membrane lots). The shaded volumes were taken from Tables 3 and 4.

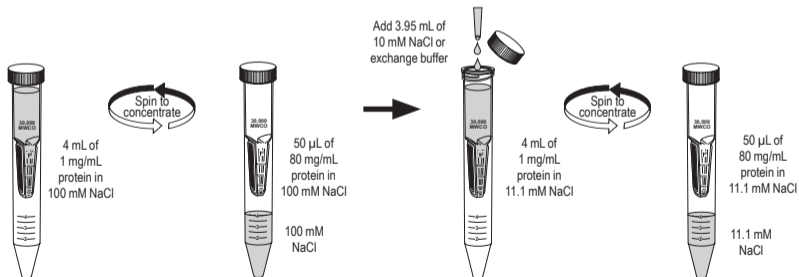
Maximizing Sample Recovery

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-4 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-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.



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
0.5	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		
4	30–70	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

Related Products

* Amicon Ultra-4 and -15 10,000 NMWL devices are for in vitro diagnostic use. All other devices are for research use only.

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 catalogue for the phone number of the office nearest you or go to our web site 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.

For matters associated with Directive 98/79/EC on in vitro diagnostic medical devices, contact the legal manufacturer:

Millipore Ireland B.V.,
Tullagreen, Carrigtwohill,
County Cork,
Ireland

In Vitro Diagnostic Product Labeling

The following table defines the symbols found on Amicon Ultra-4 10K device labels.

Symbol	Definition
	In vitro diagnostic medical device
	Catalogue number
	Do not reuse
	Use by
	Batch code
	Date of manufacture
	Manufacturer
	Temperature limitation

Standard Warranty

Millipore Corporation (“Millipore”) warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications, and descriptions of Millipore products appearing in Millipore’s published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore’s sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the customer all monies paid for such applicable product or part. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL, OR ANY OTHER INDIRECT DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

