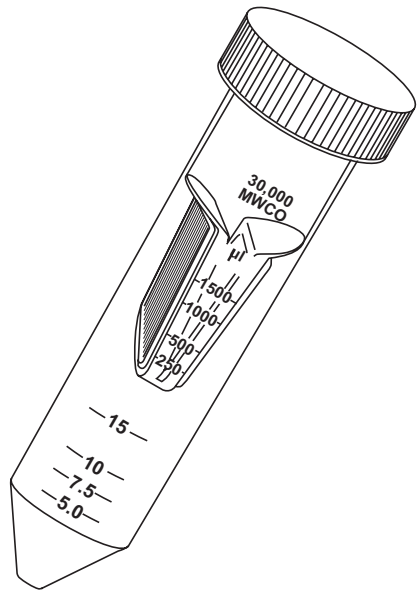
Amicon® Ultra-15

Centrifugal Filter Devices

for volumes up to 15 mL

User Guide



Amicon Ultra-15 10K device
for in vitro diagnostic use

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

Introduction, continued

The Amicon Ultra-15 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-15 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-15 3K, 30K, 50K, and 100K filter devices are for research use only and not for use in diagnostic procedures.

Applications

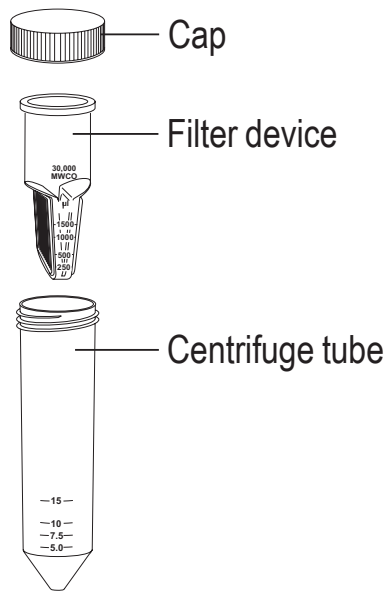
- Concentration of biological samples containing antigens, antibodies, enzymes, nucleic acids (DNA/RNA samples, either single- or double-stranded), microorganisms, column eluates, and purified samples

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-15 device is supplied with a cap, a filter device, and a 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 Quantitate Recoveries” section.

Device Storage

Store at room temperature.

Specifications

Maximum initial sample volume

Swinging bucket	15.0 mL
Fixed angle rotor	12.0 mL

Typical final concentrate volume 200 µL

Maximum relative centrifugal force

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

Active membrane area 7.6 cm^2

Dimensions

Filter device in tube (capped)

Length: 122 mm (4.81 in.) Diameter: 29.7 mm (1.17 in.)

Filter device

Length: 72.0 mm (2.84 in.) Diameter: 29.6 mm (1.17 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-15 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-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:** Spin 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 2 and 3 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

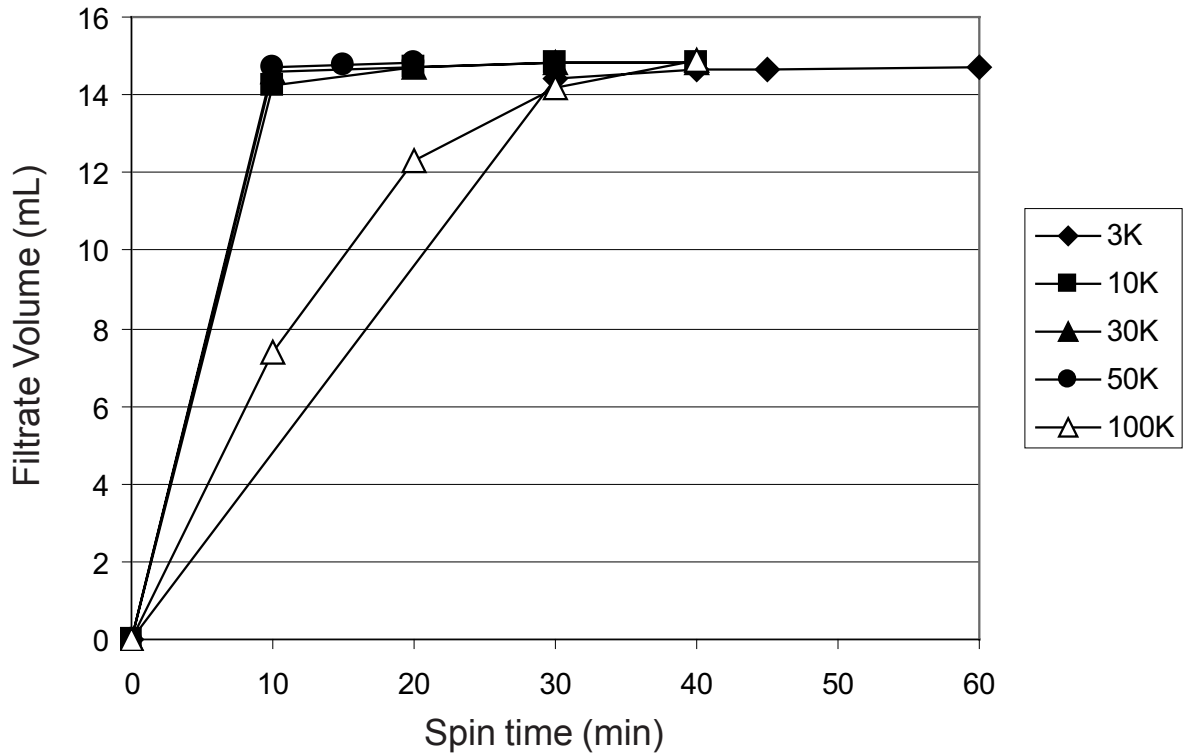
Performance - Protein Concentration

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 15 mL sample is approximately 15–60 minutes (depending on device nominal molecular weight limit). While most of the sample is filtered in the first 15 to 30 minutes of centrifugation, the lowest concentrate volume (200 µL) is reached after spinning for 15–60 minutes.

Flow rate, continued

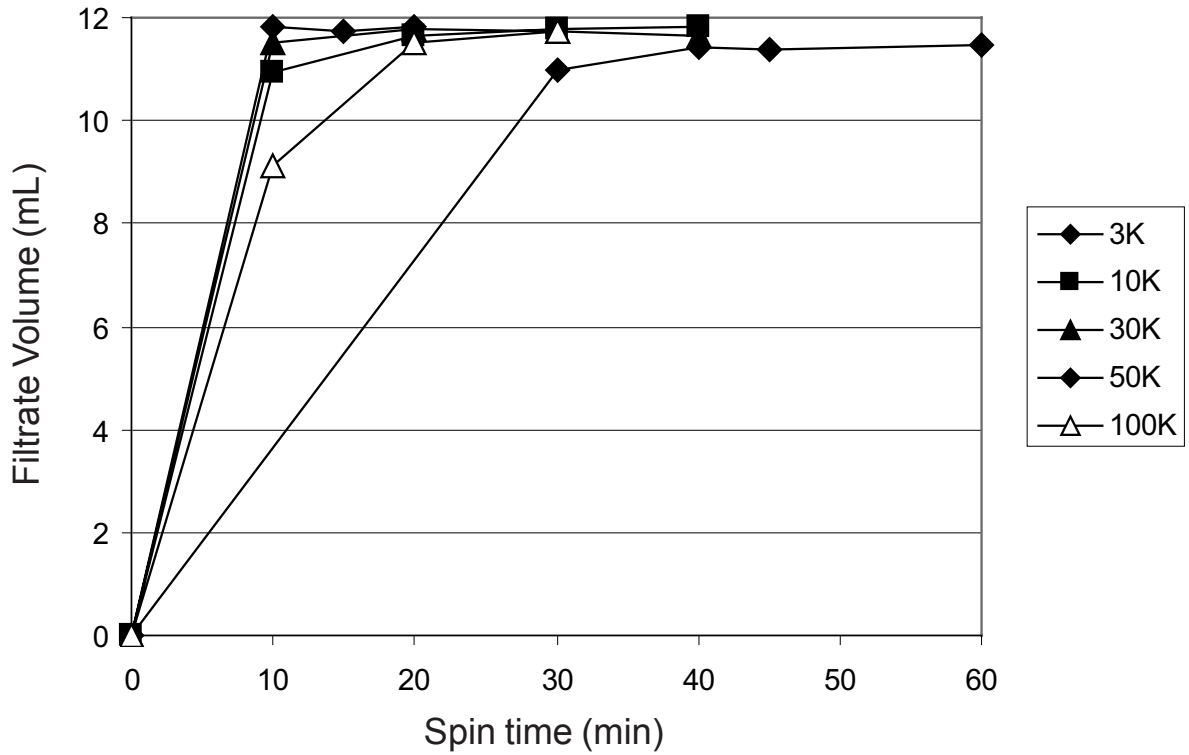
Figure 1. Typical Filtrate Volume vs. Spin Time for Amicon Ultra-15 Device (Swinging bucket rotor)



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.

Flow rate, continued

Figure 2. Typical Filtrate Volume vs. Spin Time for Amicon Ultra-15 Device (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 2: Typical Concentrate Volume vs. Spin Time (Swinging bucket rotor)

	Concentrate volume (µL)				
Spin time (min)	**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 5.

Flow rate, continued

Table 3: Typical Concentrate Volume vs. Spin Time (Fixed angle rotor)

	Concentrate volume (µL)				
Spin time (min)	**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 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. 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 4 for "Typical Retention of Protein Markers".

Protein Retention and Concentrate Recovery, continued

Table 4: 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	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

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 5 provides typical recoveries for Amicon Ultra-15 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
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 for tables 4 and 5: 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 2 and 3.

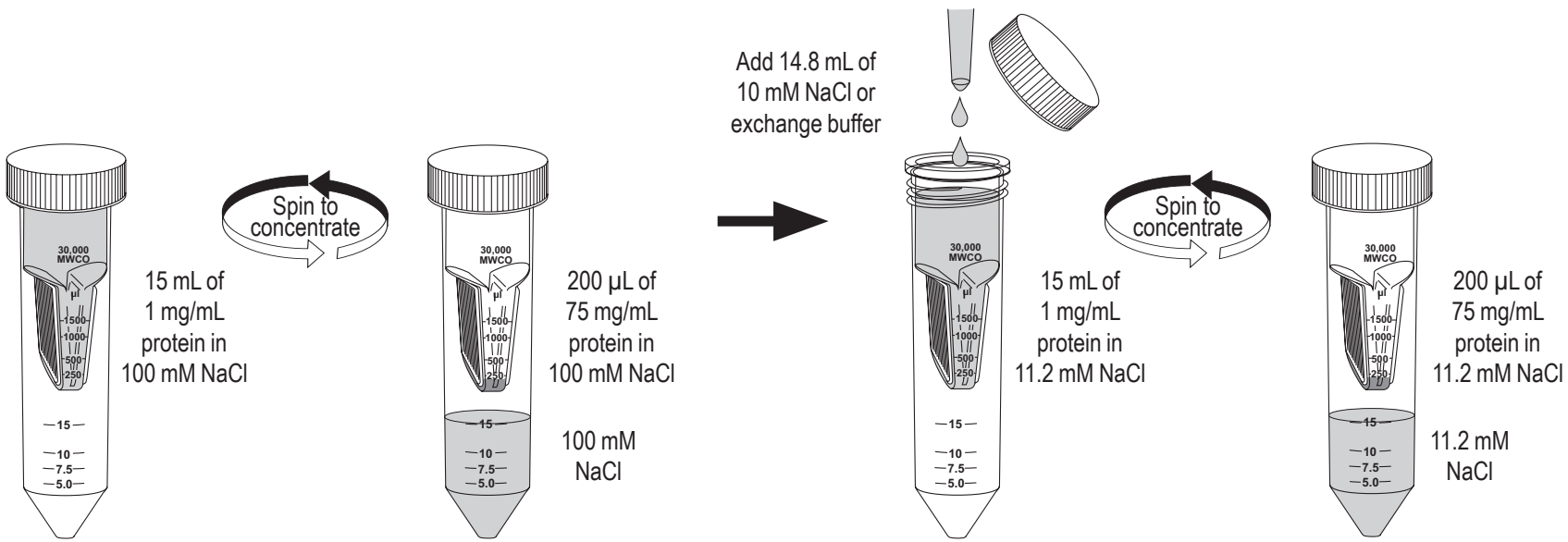
Maximizing Sample Recovery

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-15 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-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.



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 24 96 500	UFC500308 UFC500324 UFC500396 UFC5003BK	UFC501008 UFC501024 UFC501096 UFC5010BK	UFC503008 UFC503024 UFC503096 UFC5030BK	UFC505008 UFC505024 UFC505096 UFC5050BK	UFC510008 UFC510024 UFC510096 UFC5100BK
Amicon Ultra-0.5 Collection Tubes			96	UFC50VL96				
4	30–70	Amicon Ultra-4 device	8 24 96	UFC800308 UFC800324 UFC800396	UFC801008* UFC801024* UFC801096*	UFC803008 UFC803024 UFC803096	UFC805008 UFC805024 UFC805096	UFC810008 UFC810024 UFC810096
15	150–300	Amicon Ultra-15 device	8 24 96	UFC900308 UFC900324 UFC900396	UFC901008* UFC901024* UFC901096*	UFC903008 UFC903024 UFC903096	UFC905008 UFC905024 UFC905096	UFC910008 UFC910024 UFC910096

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

Related Products

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-15 10K device labels.

Symbol	Definition
IVD	In vitro diagnostic medical device
REF	Catalogue number
	Do not reuse
	Use by
LOT	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.

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