

### 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.

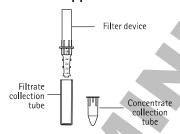
The Amicon® Ultra-2 product line includes 5 different cutoffs (Nominal Molecular Weight Limit, NMWL). These devices are for research use only and not for use in diagnostic procedures.

- 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 cluates, 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

### **Prerinsing**

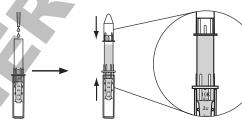
The ultrafiltration membranes in Amicon® Ultra-2 devices contain trac material interferes with analysis, pre-rinse the device with buffer or Mi continues, rinse with 0.1 N NaOH followed by a second spin of buffer o

CAUTION: Do not allow the membrane in Amicon® Ultra filter devices to using the device immediately after pre-rinsing, leave fluid on is used.

### How to Use Amicon® Ultra-2 Centrifugal Filt

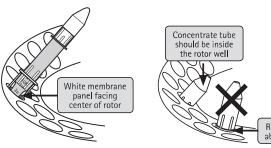
- Insert the Amicon® Ultra-2 device into the filtrate collection tube seated in the tube.
- Add up to 2 mL of sample to the device and cover with concentrat firmly onto the device.

WARNING: Failure to fully seat the device in the filtrate collection collection tube firmly onto the device may result in th centrifugation. See figure below.



Add sample Make sure both tubes are fully seated o

3. Place filter device into the centrifuge rotor with one membrane pa (one panel facing up and the other panel facing down). Make sure of the rotor well and that the rim of the concentrate collection tul figures below. Counterbalance with a similar device.



Orient device correctly in rotor

Seat device fully in well

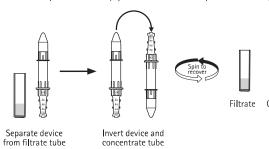
- 4. Spin for approximately 10–60 minutes depending on the NMWL o  $4,\!000\times g \ maximum \ when \ using \ a \ swinging \ bucket \ rotor$ 
  - 7,500 × g maximum when using a fixed angle rotor

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

Refer to Figures 1 and 2, and Tables 2 and 3 for typical spin times.

- Remove the assembled device from the centrifuge and separate th the filtrate collection tube.
- To recover the concentrated solute, invert the Amicon® Ultra filter tube. Place in centrifuge and counterbalance with a similar device 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 solutions containing biomolecules. The removal of salts or the exchang in the Amicon® Ultra-2 device by concentrating the sample, discarding the concentrate to the original sample volume with any desired solvent be repeated until the concentration of the contaminating microsolute example below.

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

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

3K device		TOK device		30K device		50K device		TOOK 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:  $35^\circ$  fixed angle rotor,  $7,500 \times g$ , room temperature, 2 mL starting volume. Protein markers used: Cytochrome c for 3K and 10K, BSA for 30K and 50K, and 1gG 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
lgG (1 mg/mL)	156,000		95		95	
Ovalbumin (1 mg/mL)	45,000		12		13	

Spin Conditions: Swinging bucket rotor, 4,000  $\times$  g, or 35° fixed angle rotor, 7,500  $\times$  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

		Spin Time (min)		Concentrate Volume (µL)		Concentration Factor (x)		Concentrate Recovery (%)	
Marker/ Concentration	Device NMWL	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,  $4,000 \times g$ , or 35° fixed angle rotor,  $7,500 \times g$ , 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.

### How to Quantify Recoveries

Calculate total recovery, percent concentrate recovery, and percent filt below. The procedure provides a close approximation of recoveries for sto roughly 20 mg/mL.

NOTE: Appropriate assay techniques include absorption spectrophotor index, and conductivity.

### **Direct Weighing Procedure**

The density of most dilute proteins is nearly equal to the density of war the concentrate and filtrate volumes can be quantified by weighing the grams to milliliters. This technique is valid only for solutions with conce 20 mg/mL or less.

- Separately weigh the empty filter device, filtrate collection tube, a before use.
- 2. Fill filter device with solution and reweigh.
- . Assemble device in filtrate collection tube and centrifuge per insti
- I. Collect the concentrate by reverse spin into the pre-weighed conc
- Remove the device from the concentrate collection tube and weig collection tubes.
- Subtract weight of empty device/tubes to calculate weights of sta concentrate.
- 7. Assay the starting material, filtrate, and concentrate to determine
- 3. Calculate recoveries using the weight/volume data and the measu

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

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

% total recovery = % concentrate recovery + % filtrate recovery

W<sub>c</sub> = total weight of concentrate before assay

W₀ = weight of original starting material

W<sub>f</sub> = weight of filtrate

 $C_c$  = concentrate concentration

 $C_o =$  original starting material concentration

C<sub>f</sub> = 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  $4,000 \times g$  for concentration spin Fixed angle rotor  $7,500 \times g$  for concentration spin

NOTE: When spinning viscous so or plasma, do not exceed

Active membrane area  $1 \text{ cm}^2$ Hold-up volume  $< 5 \text{ }\mu\text{L}$ 

Dimensions

Filter device and tube

Length (concentration mode; device in filtrate tube): 119.7

Length (recovery spin; device upside down in concentrate the Filter device Diameter: 15.9 mm (0.63 in.) Length Filtrate tube Diameter: 13.8 mm (0.54 in.) Length Concentrate tube Diameter: 13.7 mm (0.54 in.) Length Concentrate tube

Concentrate tube

Materials of Construction

Filter device Copolymer styrene/butadiene
Membrane Ultracel® low binding regenerate
Collection tubes Polypropylene

### **Chemical Compatibility**

Amicon® Ultra centrifugal devices are intended for use with biological use, check the sample for chemical compatibility with the device.

Table 6. Chemical Compatibility of Amicon® Ultra Filter Devices

	Acids	Concentration	
	Acetic acid	≤ 50%*	Phosphoric acid
	Formic acid	≤ 5%*	Sulfamic acid
ĺ	Hydrochloric acid	≤ 1.0 M	Sulfuric acid
	Lactic acid	≤ 50%	Trichloroacetic ac