

Flow Rate, continued

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

Spin Time (min)	3K device		10K device		30K device		50K device		100K device	
	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.

How to Quantify Recoveries

Calculate total recovery, percent concentrate recovery, and percent filtrate recovery below. The procedure provides a close approximation of recoveries for solutions with concentrations roughly 20 mg/mL.

NOTE: Appropriate assay techniques include absorption spectrophotometry, protein assay, and conductivity.

Direct Weighing Procedure

The density of most dilute proteins is nearly equal to the density of water. Therefore, the concentrate and filtrate volumes can be quantified by weighing the samples in grams to milliliters. This technique is valid only for solutions with concentrations 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 collection tubes.
6. Subtract weight of empty device/tubes to calculate weights of starting material and concentrate.
7. Assay the starting material, filtrate, and concentrate to determine concentrations.
8. Calculate recoveries using the weight/volume data and the measured concentrations.

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

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

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

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

Fixed angle rotor 7,500 × g for concentration spin

NOTE: When spinning viscous samples or plasma, do not exceed 2,000 × 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

Length (recovery spin; device upside down in concentrate tube): 119.7 mm

Filter device Diameter: 15.9 mm (0.63 in.) Length: 119.7 mm

Filtrate tube Diameter: 13.8 mm (0.54 in.) Length: 119.7 mm

Concentrate tube Diameter: 13.7 mm (0.54 in.) Length: 119.7 mm

Materials of Construction

Filter device Copolymer styrene/butadiene

Membrane Ultracef® low binding regenerated cellulose

Collection tubes Polypropylene

Chemical Compatibility

Amicon® Ultra centrifugal devices are intended for use with biological samples. Before 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
Hydrochloric acid	≤ 1.0 M	Sulfuric acid
Lactic acid	≤ 50%	Trichloroacetic acid
Nitric acid	≤ 10%	