



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). Solvent 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.

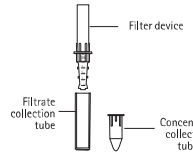
The Amicon® Ultra-2 product line includes 5 different cutoffs (Nominal Molecular Weight Limit, NMWL). These devices are for research use only and not for use in diagnostic procedures.

- 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 x 100 mm tubes (same well/carrier size as for Amicon® Ultra-4 devices and the former Centricron® 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

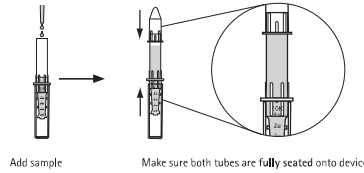
The ultrafiltration membranes in Amicon® Ultra-2 devices contain trace amounts of glycerine. If this material interferes with analysis, pre-rinse the device with buffer or Milli-Q® water. If interference continues, rinse with 0.1 N NaOH followed by a second spin of buffer or Milli-Q® water.

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.

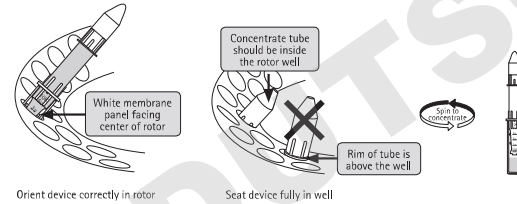
How to Use Amicon® Ultra-2 Centrifugal Filter Devices

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 x g maximum when using a swinging bucket rotor

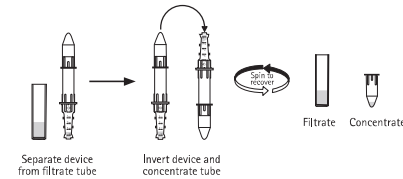
7,500 x g maximum when using a fixed angle rotor

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.

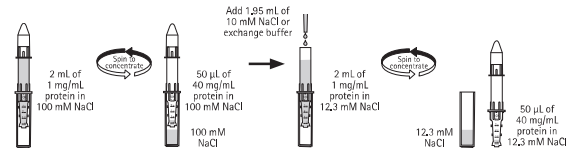
5. Remove the assembled device from the centrifuge and separate the Amicon® Ultra filter device from the filtrate collection tube.
6. To recover the concentrated solute, invert the Amicon® Ultra filter device and concentrate collection tube. Place in centrifuge and counterbalance with a similar device. Spin for 2 minutes at 1,000 x 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 microsolvent 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

PCR Product (base pairs)	PCR Primer (bases)	Swinging Bucket Rotor 4,000 x g for 40 min			35° Fixed Angle Rotor 7,500 x g, for 15 min		
		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

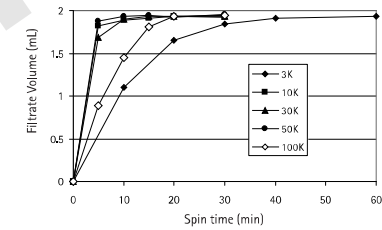
100 µL PCR diluted to 2,000 µL starting volume, n=6

Performance – Protein Concentration

Flow Rate

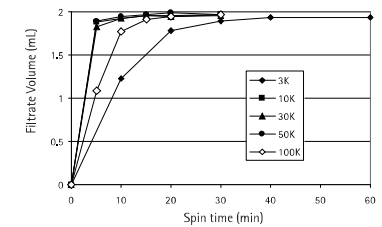
Factors affecting flow rate include sample concentration, starting volume, chemical nature of solute, relative centrifugal force, centrifuge rotor angle, membrane type, and temperature. Figures 1 and 2 and Tables 2 and 3 can be used to estimate the time required to achieve a given volume of filtrate 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 x 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 x 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

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					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 x 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.

