Macrosep™ Advance centrifugal devices

Instructions for Use

Introduction

Intended use

Cytiva Lab Filtration products are designed for professional laboratory applications only. These products are not approved for use in medical, clinical, surgical or other patient protection applications. They are also not suitable for use in Biopharmaceutical manufacturing or production.

Important

Employment of the products in applications not specified, or failure to follow all instructions contained in this Instructions for Use, can result in personal injury, damage to property or the product, or improper functioning of the product.

Background

Description

Macrosep™ Advance centrifugal devices:

- Concentrate and purify samples up to 20 mL.
- Provides recoveries typically > 90%.
- Built-in deadstop prevents spinning to dryness.

Macrosep Advance centrifugal devices provide rapid and efficient concentration and purification of up to 20 mL of biological samples. The unique design maximizes filtration area to process samples quickly while maintaining a gentle concentration environment to preserve protein activity and conformation. The wide selection of ultrafiltration molecular weight cut-off (MWCO) devices incorporate Omega™ membrane which is very low in protein and nucleic acid binding.

Ultrafiltration devices are ideal for concentrating small peptides, oligonucleotides, nucleic acids, enzymes, antibodies, and other similar macromolecules. Macrosep Advance centrifugal filters are also available in 0.2 and 0.45 µm pore sizes containing Supor™ polyethersulfone membrane for low protein and nucleic acid binding with high chemical compatibility. The microporous membrane selections are ideal for microorganism concentration, sample clarification, removal of particulates and colloids, and gentle elution of nucleic acid from agarose gels.

Filtration principles for Macrosep Advance centrifugal devices

Centrifugation provides the driving force for filtration. Ultrafiltration devices are typically centrifuged between 1000 to 5000 × g. Biomolecules larger than the nominal MWCO of the membrane are retained in the sample reservoir while solutions and low molecular weight molecules pass through the membrane into the filtrate receiver. See the following table for recommendations on how to choose the correct MWCO for your application. Microfiltration membrane devices can be centrifuged up to 14 000 × g. Similarly, particulate larger than the membrane pore size are retained in the sample reservoir while solutions and particulate smaller than the pore size pass through into the filtrate receiver.

Applications

Macrosep Advance centrifugal devices with ultrafiltration membrane can be used for:

- Concentrate and desalt proteins and nucleic acids.
- Buffer exchange or salt removal of chromatography fractions.
- Harvest biomolecules from cell culture media.
- Virus concentration or removal.
- Crude fractionation of protein mixtures.
- Remove debris and particulate from cell lysates.

Macrosep Advance centrifugal devices with microfiltration membrane can be used for:

- Separate DNA from agarose gels.
- Separate proteins, oligonucleotides, and RNA from polyacrylamide gels.
- Clarify samples before HPLC analysis.
- Remove cells from media prior to analysis.
- Filtration of biological samples.
- Collect and wash treated particles or beads.
- Fill with a chromatographic medium for analytical procedures or process development.

Choosing the appropriate Macrosep Advance centrifugal device for ultrafiltration applications

Protein applications

For maximum retention, select a Macrosep Advance centrifugal device with a MWCO 3 to 6 times less than the molecular weight of the protein to be retained. For example, for a 150K protein, a 30K Macrosep Advance centrifugal device would be the appropriate selection.

DNA applications

The molecular weight of a strand of DNA can be estimated by multiplying the number of bases by 340 for single stranded DNA, and the number of base pairs by 680 for double stranded DNA. Once the molecular weight of the DNA is estimated, select a Macrosep Advance centrifugal device with a molecular weight cutoff 3 to 6 times less than the molecular weight of the DNA to be retained. For example, to retain a 2 kilobase (Kb) double stranded DNA fragment: 2000 × 680 = 1 360 000 Daltons = 1360K Daltons; a 100K Macrosep Advance centrifugal device would be the appropriate selection.

The table below is a guide for initial selection of Macrosep Advance centrifugal devices MWCOs for retention of proteins and nucleic acids. Ionic conditions, molecular conformation, and protein:protein interactions can affect retention of biomolecules. We recommend pretesting retentivity with your biomolecular solution.

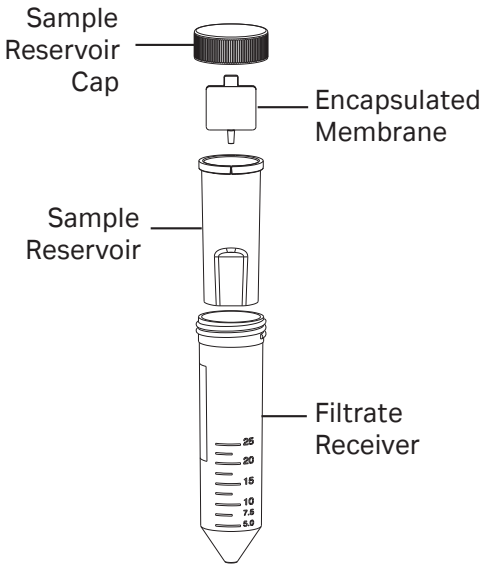
Table 1. Macrosep Advance selection

Macrosep Advance MWCO	Recommended g-force	Biomolecule molecular weight or size	Nucleic acid	
			Base pair (ds)	Bases (ss)
1K	3000 to 5000 × g	3K to 10K	5 to 16 bp	9 to 32 bs
3K	3000 to 5000 × g	10K to 30K	16 to 50 bp	32 to 95 bs
10K	3000 to 5000 × g	30K to 90K	50 to 145 bp	95 to 285 bs
30K	3000 to 5000 × g	90K to 300K	145 to 475 bp	285 to 950 bs
100K	1000 to 3000 × g	> 300K	475 to 1450 bp	950 to 2900 bs

Components

Each Macrosep Advance centrifugal device consists of a screw-on cap, sample reservoir containing a paddle with sealed membrane on both sides, and a filtrate receiver tube.

The insert and standard centrifuge tube design provides maximum stability for handling and centrifugation. The filtrate receiver tube provides graduations to measure buffer and samples plus a large area to clearly label sample identification.



Color-coding

Each MWCO for the Macrosep Advance centrifugal device is color-coded for easy identification.

MWCO	Color
1K	Yellow
3K	Gray
10K	Blue
30K	Red
100K	Clear
0.2 µm	Aqua
0.45 µm	Wildberry and clear

Operating Instructions

Pre-rinsing (optional)

For the majority of applications, Macrosep Advance centrifugal devices can be used without pre-rinsing. However, under certain conditions, it may be preferable to remove trace extractables.

Microfiltration devices: Contact with some organic solvents may cause materials to leach out from the device components. If these leachables represent potential assay interferences, they may be removed by filtering 20 mL of the solvent to be used in the application at 14 000 × g for 1 minute. Discard filtrate and repeat.

Ultrafiltration devices: Omega membrane contains trace amounts of glycerine and sodium azide. If these chemicals interfere with an assay, they may be removed by filtering 20 mL deionized water or buffer through the membrane and repeat. If further flushing is required, start with 0.05 N NaOH and repeat this procedure. Use the device within 20 minutes to prevent irreversible membrane damage due to dehydration.

Instructions for use

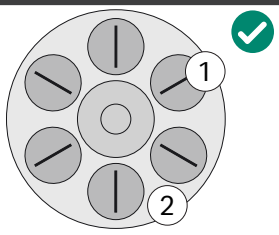
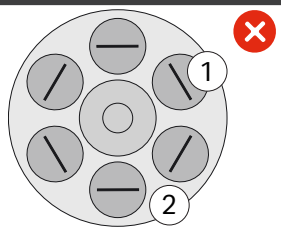
Step	Action
1	Remove cap and pipette 5 to 20 mL sample into sample reservoir and replace cap to prevent evaporation during centrifugation.
2	Place device into centrifuge that accepts 50 mL conical-end tubes. Always counterbalance the rotor with another Macrosep Advance centrifugal device containing the equivalent sample volume. To make sure of correct placement of the centrifugal devices, see *Orientation, on page 3*.
3	Spin device at recommended force for required time. • Ultrafiltration: Spin at 1000 to 5000 × g, typically for 30 to 90 minutes, to achieve desired concentrate volume. It is recommended that spin time and g-force be determined for each application. • Microfiltration: Spin at up to 14 000 × g for 1 to 3 minutes.
4	Remove the device from the centrifuge and recover target of interest retained in sample reservoir or filtrate receiver tube. • Target of interest in the sample reservoir: Use pipette to transfer concentrated sample to microcentrifuge tube for storage.

Step	Action
	• Target of interest in the filtrate receiver: Remove and discard the sample reservoir and tightly cap the filtrate receiver for storage.

Orientation

Orient the Macrosep Advance centrifugal device correctly for effective filtration. The vertical paddle containing the encapsulated membrane must always be placed perpendicular to the center rotor of the centrifuge. This allows for sufficient contact between the membrane and the sample.

The table below shows Macrosep Advance centrifugal devices (1) that have been placed correctly and incorrectly in the fixed angle rotor (2).

Correct orientation	Incorrect orientation
	

Non-specific adsorption

Omega membranes are made from polyethersulfone specifically modified to minimize protein binding. These membranes provide equivalent or higher recoveries than comparable regenerated cellulose membranes and offer exceptional biological and chemical resistance.

Adsorption to device components is of particular concern when purifying microgram or nanogram levels of protein. Even with the advanced plastics used in Macrosep Advance centrifugal devices, some adsorption may occur with particularly "sticky" proteins and biomolecules. Pre-treating Macrosep Advance centrifugal devices may further reduce non-specific adsorption to the device.

Step	Action
1	Fill sample reservoir with 20 mL of 10% glycerine.
2	Soak overnight at room temperature.
3	Rinse the device with deionized water.
4	Fill the sample reservoir with 20 mL of deionized water and spin. Repeat.
5	Use device as normal.

Diafiltration (desalting and buffer exchange)

For salt removal or buffer exchange:

Step	Action
1	Concentrate the sample at least tenfold (e.g., 20 mL concentrated to 2 mL).
2	Reconstitute with exchange buffer and reconcentrate tenfold.
3	Repeat this procedure 3 to 5 times to remove 95% to 99% of salt or buffer.

Optimization

Factors affecting performance

Variations in flow rates and recovery can be caused by the following:

- Protein concentration (Macrosep Advance centrifugal devices perform optimally at 1 mg/mL or less protein).
- Temperature (slower flow rates occur at colder temperatures).
- Protein:protein interactions that may cause retention of molecules that would normally pass through the membrane.
- Ionic conditions.
- Size or conformation of the molecule.

Table 2. Effects of centrifugal force on concentration times

MWCO	Solute	Time to 25 × concentration (min)		
		1000 × g	3000 × g	5000 × g
1K	Ubiquitin (0.25 mg/mL)	>180 min	>180 min	165 min
3K	Cytochrome C (0.25 mg/mL)	720	300	180
10K	Albumin (1 mg/mL)	150	60	45
30K	IgG (1 mg/mL)	90	60	45
100K	Thyroglubulin (1 mg/mL)	90	60	30

Table 3. Effects of starting protein concentration

MWCO	Solute	Time to 25 × concentration (min)
1K	Ubiquitin 1 mg/mL	105
	Ubiquitin 0.5 mg/mL	105
	Ubiquitin 0.1 mg/mL	105
3K	Cytochrome C 1 mg/mL	210
	Cytochrome C 0.5 mg /mL	180
	Cytochrome C 0.1 mg/mL	120
10K	Albumin 1 mg/mL	45
	Albumin 0.5 mg /mL	45
	Albumin 0.1 mg/mL	45
30K	IgG 1 mg/mL	45
	IgG 0.5 mg /mL	30
	IgG 0.1 mg/mL	30
100K	Thyroglubulin 1 mg/mL	30
	Thyroglubulin 0.5 mg /mL	30
	Thyroglubulin 0.1 mg/mL	30

Specifications

Parameter	Specification	
Materials of construction	Filtration media	Ultrafiltration: Omega membrane (modified polyethersulfone)
		Microfiltration: Supor membrane (polyethersulfone)
	Sample Reservoir, filtrate receiver, and cap	Polypropylene
	Paddle	Polyethylene
Effective filtration area	7.2 cm^2 (1.12 $in.^2$)	
Dimensions	Diameter	29 mm (1.2 in.)
	Length	12.0 cm (4.7 in.)
Capacities	Maximum sample volume	20 mL
	Maximum filtrate receiver volume	22 mL
	Hold-up volume	80 µL
	Dead stop volume	34° fixed angle 1.5 mL
		45° fixed angle 1.2 mL
		Swinging bucket 450 µL
Operating temperature range	0°C to 40°C (32 to 104 °F)	
pH range	Ultrafiltration	2 to 14
	Microfiltration	1 to 14
Maximum centrifugal force	Ultrafiltration	5000 × g
	Microfiltration	14 000 × g
Centrifuge	Fits centrifuges that accept standard 50 mL conical-end tubes	
Sanitization	Provided non-sterile, may be sanitized by filtering 70% ethanol through the device prior to use.	

Ordering information

Description	6/pkg	24/pkg	100/pkg
1K Omega	MAP001C36	MAP001C37	MAP001C38
3K Omega	MAP003C36	MAP003C37	MAP003C38
10K Omega	MAP010C36	MAP010C37	MAP010C38
30K Omega	MAP030C36	MAP030C37	MAP030C38
100K Omega	MAP100C36	MAP100C37	MAP100C38
0.2 µm Supor	-	MAPM02C67	MAPM02C68
0.45 µm Supor	-	MAPM45C67	MAPM45C68

Complementary products

- Cytiva offers centrifugal devices for processing the following sample volumes:

Device	Sample volume
Nanosep™ Device	up to 0.5 mL
Microsep™ Advance Device	up to 5 mL
Macrosep Advance Device	up to 20 mL
Jumbosep™ Device	up to 60 mL

- Minimate™ tangential flow filtration devices are typically used for the concentration or diafiltration of 100 mL to 5 liter samples.
- AcroPrep™ Advance 96-well filter plates with Supor and Omega membranes exhibit low binding capacities for protein and nucleic acid purification.
- Filtration devices with Supor membrane are sterile, ready-to-use, and maximize sample recoveries with low protein-binding membrane and low hold-up volumes.

Give feedback on this document

Visit cytiva.com/techdocfeedback or scan the QR code.

cytiva.com

For local office contact information, visit cytiva.com/contact

89113 AA V:11 01/2025