PALL Life Sciences

Macrosep® Advance Centrifugal Devices

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

Ordering Information

Macrosep Advance Centrifugal Devices

Description	6/pkg	24/pkg	100/pkg
1K Omega™, Yellow	MAP001C36	MAP001C37	MAP001C38
3K Omega, Gray	MAP003C36	MAP003C37	MAP003C38
10K Omega, Blue	MAP010C36	MAP010C37	MAP010C38
30K Omega, Red	MAP030C36	MAP030C37	MAP030C38
100K Omega, Clear	MAP100C36	MAP100C37	MAP100C38
0.2 µm Supor®, Aqua	—	MAPM02C67	MAPM02C68
0.45 µm Supor, Wildberry	—	MAPM45C67	MAPM45C68

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Note: *The procedures herein are intended only as a guide. Users should always verify product performance with their specific applications under actual use conditions. If you have questions about the information presented in this guide, please contact our Technical Service department.*

Introduction

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 Pall's 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 1,000 to 5,000 x 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. Microfiltration membrane devices can be centrifuged up to 14,000 x 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.

Specifications

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:
- 34º fixed angle 1.5 mL
- 45º fixed angle 1.2 mL
- Swinging bucket 450 µL

Operating Temperature Range
0-40 ºC (32-104 ºF)

pH Range
Ultrafiltration: 1-14
Microfiltration: 1-14

Maximum Centrifugal Force
Ultrafiltration: 5,000 x g
Microfiltration: 14,000 x 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.

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 x 680 = 1,360,000 Daltons = 1360K Daltons; a 100K Macrosep Advance centrifugal device would be the appropriate selection.

Choosing the Appropriate Macrosep Advance Centrifugal Device for Ultrafiltration Applications *(continued)*

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 Device MWCO	Recommended g-force	Biomolecule Molecular Weight or Size	Nucleic Acid	
			Base Pair (ds)	Bases (ss)
1K	3,000-5,000 x g	3K-10K	5-16 bp	9-32 bs
3K	3,000-5,000 x g	10K-20K	16-32 bp	32-65 bs
10K	3,000-5,000 x g	30K-90K	50-145 bp	95-285 bs
30K	3,000-5,000 x g	90K-180K	145-285 bp	285-570 bs
100K	1,000-3,000 x g	> 300K	475-1,450 bp	950-2,900 bs

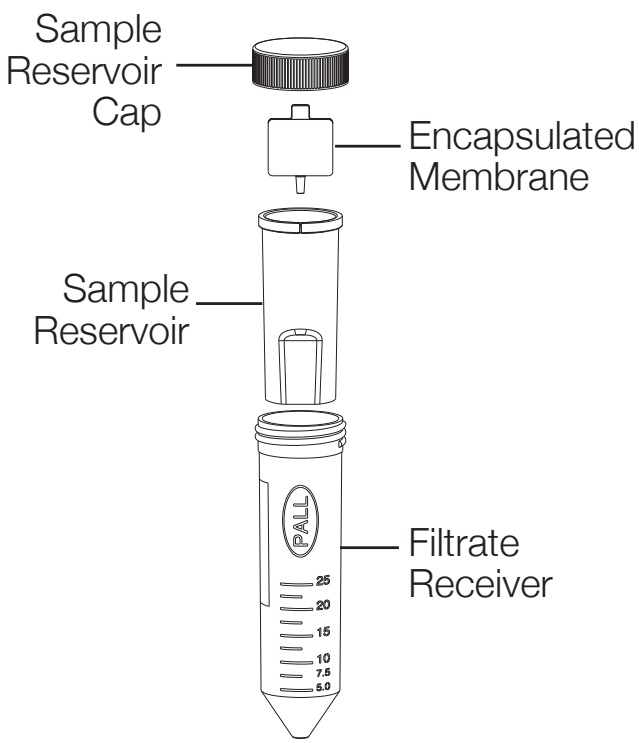
Components

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.

Figure 1

Macrosep Advance Centrifugal Device



Macrosep Advance Centrifugal Device Operation

Instructions for Use

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.
3. Spin device at recommended force for required time.
   a. Ultrafiltration: Spin at 1,000 to 5,000 x 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.
   b. Microfiltration: Spin at up to 14,000 x g for 1-3 minutes.
4. Remove the device from the centrifuge and recover target of interest retained in sample reservoir or filtrate receiver tube.
   a. Target of interest in the sample reservoir: Use pipette to transfer concentrated sample to microcentrifuge tube for storage.
   b. Target of interest in the filtrate receiver: Remove and discard the sample reservoir and tightly cap the filtrate receiver for storage.

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 x 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 0.05N NaOH and repeat this procedure. Use the device within 20 minutes to prevent irreversible membrane damage due to dehydration.

Macrosep Advance Centrifugal Device Operation *(continued)*

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.

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.

Macrosep Advance Centrifugal Device Operation *(continued)*

Diafiltration (Desalting and Buffer Exchange)

For salt removal or buffer exchange:

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; and size or conformation of the molecule.

Macrosep Advance Centrifugal Device Operation *(continued)*

Table 2. Effects of Centrifugal Force on Concentration Times

		Time to 25x Concentration (min)		
MWCO	**Solute**	**1,000 x g**	**3,000 x g**	**5,000 x 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

Macrosep Advance Centrifugal Device Operation *(continued)*

Table 3. Effects of Starting Protein Concentration

MWCO	Solute	Time to 25x 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

Complementary Products

- Pall Life Sciences 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.
- **BioTrace™ and Biodyne® Transfer Membranes** offer precise performance and compatibility with nearly every detection system available.
- **AcroPrep™ and 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.

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