PALL Life Sciences

Microsep™ Advance Centrifugal Devices

- Concentrate and purify samples up to 5 mL.
- Provides recoveries typically > 90%.
- Built-in deadstop prevents spinning to dryness.

Ordering Information

Microsep Advance Centrifugal Devices

Description	24/pkg	100/pkg
1K Omega™, Yellow	MCP001C41	MCP001C46
3K Omega, Gray	MCP003C41	MCP003C46
10K Omega, Blue	MCP010C41	MCP010C46
30K Omega, Red	MCP030C41	MCP030C46
100K Omega, Clear	MCP100C41	MCP100C46
0.2 µm Supor®, Aqua	MCPM02C67	MCPM02C68
0.45 µm Supor, Wildberry	MCPM45C67	MCPM45C68

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Table of Contents

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

Microsep Advance centrifugal devices provide rapid and efficient concentration and purification of up to 5.0 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. Microsep 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 Microsep Advance Centrifugal Devices

Centrifugation provides the driving force for filtration. Ultrafiltration devices are typically centrifuged between 3,000 to 7,500 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 (0.2, 0.45 µm): Supor membrane (polyethersulfone)

Sample Reservoir, Filtrate Receiver, and Cap: Polypropylene
Paddle: Polyethylene

Effective Filtration Area
3.3 cm^2 (0.5 $in.^2$)

Dimensions
Diameter: 17 mm (0.7 in.)
Length: 12.0 cm (4.9 in.)

Capacities
Maximum Sample Volume: 5 mL
Maximum Filtrate Receiver Volume: 6.5 mL
Hold-up Volume: 40 µL
Dead Stop Volume:
- 34º fixed angle 100 µL
- 45º fixed angle 80 µL
- Swinging bucket 65 µL

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

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

Maximum Centrifugal Force
Ultrafiltration: 7,500 x g
Microfiltration: 14,000 x g

Centrifuge
Fits centrifuges that accept standard 17 x 100 mm conical-end tubes

Sanitization
Provided non-sterile, may be sanitized by filtering 70% ethanol through the device prior to use.

Applications

Microsep Advance centrifugal devices with ultrafiltration membrane replace dialysis, chemical precipitation, and lyophilization in the following applications:

- Concentrate and desalt proteins and nucleic acids
- Buffer exchange or salt removal of chromatography fractions
- Deprotein serum or urine for HPLC analysis of drugs, amino acids, and antibiotics
- Recover biomolecules from cell culture supernatants or lysates
- Isolation of low molecular weight compounds from fermentation broths for natural product screening
- Separate primers from amplified DNA product
- Purify hybridization probes or remove unincorporated nucleotides

Microsep Advance centrifugal devices with microfiltration membrane can be used to:

- 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
- Filter biological samples
- Collect and wash treated particles or beads
- Fill with a chromatographic medium for analytical procedures or process development

Choosing the Appropriate Microsep Advance Centrifugal Device for Ultrafiltration Applications

Protein Applications

For maximum retention, select a Microsep 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 Microsep 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 Microsep Advance centrifugal device with a MWCO 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 Microsep Advance centrifugal device would be the appropriate selection.

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

The table below is a guide for initial selection of Microsep Advance centrifugal device 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. Microsep Advance Selection

Microsep Advance Device MWCO	Recommended g-force	Biomolecule Molecular Weight or Size	Nucleic Acid	
			Base Pair (ds)	Bases (ss)
1K	5,000-7,500 x g	3K-10K	5-16 bp	9-32 bs
3K	5,000-7,500 x g	10K-20K	16-32 bp	32-65 bs
10K	5,000-7,500 x g	30K-90K	50-145 bp	95-285 bs
30K	5,000-7,500 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

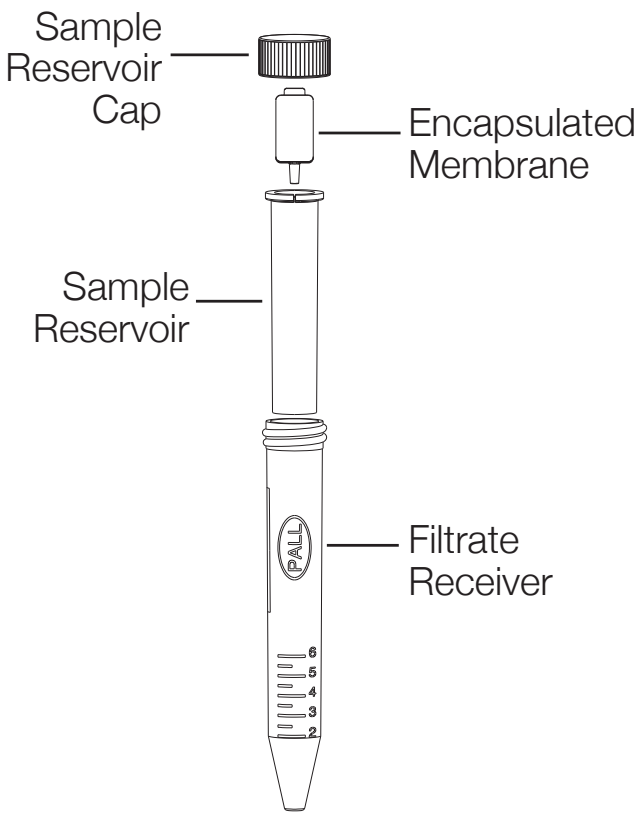
Components

Each Microsep 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

Microsep Advance Centrifigal Device



Microsep Advance Centrifugal Device Operation

Instructions for Use

1. Remove cap and pipette 0.1 to 5 mL sample into sample reservoir and replace cap to prevent evaporation during centrifugation.
2. Place device into centrifuge that accepts 17 x 100 mm conical-end tubes. Always counterbalance the rotor with another Microsep Advance centrifugal device containing the equivalent sample volume.
3. Spin device at recommended force for required time.
   a. Ultrafiltration: Spin at 3,000 to 7,500 x g for the required length of time, typically 30 to 90 minutes to achieve desired concentrate volume. For optimal performance, it is recommended that spin time and g-force be determined for each application. See Table 2 and Table 3 (pages 10 and 11) to determine appropriate protocol.
   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.

Microsep Advance Centrifugal Device Operation *(continued)*

Pre-Rinsing (Optional)

For the majority of applications, Microsep 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 from the device components. If these leachables represent potential assay interferences, they may be removed by filtering 5 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 5 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.

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 Microsep Advance centrifugal devices, some adsorption may occur with particularly “sticky” biomolecules. Pretreating Microsep Advance centrifugal devices may further reduce non-specific adsorption to the device.

1. Fill reservoir with 5 mL 10% glycerine.
2. Soak overnight at room temperature.
3. Rinse with deionized H_2O.
4. Fill with deionized H_2O and spin. Repeat.

Use device within 20 minutes to prevent irreversible membrane damage due to dehydration.

Microsep Advance Centrifugal Device Operation *(continued)*

Diafiltration (Desalting and Buffer Exchange)

For salt removal or buffer exchange, first concentrate sample at least 10-fold (i.e. 1 mL concentrated to 100 µL). Reconstitute with exchange buffer and reconcentrate 10-fold. Repeat this procedure 3 to 5 times to remove 95 to 99% of salt or buffer.

Sample Preparation for SDS-PAGE Electrophoresis

Microsep Advance devices can simplify sample preparation prior to SDS-PAGE electrophoresis.

1. Pipette 50 to 100 µL sample containing 5 to 60 µg of protein into Microsep Advance centrifugal device.
2. Dilute sample to 5 mL with buffer. Spin to deadstop. Repeat twice.
3. Transfer concentrated sample to microcentrifuge tube. Add SDS. Cap cup and heat to 80 °C for 10 minutes or more.
4. Remove from incubator or water bath and add dithiothreitol. Incubate at 56 °C.
5. Remove from incubator. Cool to room temperature. Prepare for layering on gel.

(Based on Laemmli, U.K., Nature 227, 680-685, 1970.)

Microsep Advance Centrifugal Device Operation *(continued)*

Optimization
Factors Affecting Performance

Variations in flow rates and recovery can be caused by the following: protein concentration (Microsep 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.

Table 2. Effects of Centrifugal Force on Concentration Times

		Time to 25x Concentration (min)		
MWCO	**Solute**	**3,000 x g**	**5,000 x g**	**7,500 x g**
1K	Ubiquitin (0.25 mg/mL)	90	90	75
3K	Cytochrome C (0.25 mg/mL)	75	75	75
10K	Albumin (1 mg/mL)	20	15	10
30K	IgG (1 mg/mL)	20	15	15
100K	Thyroglubulin (1 mg/mL)	15	15	10

Microsep 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	75
	Ubiquitin 0.1 mg/mL	60
3K	Cytochrome C 1 mg/mL	60
	Cytochrome C 0.5 mg /mL	60
	Cytochrome C 0.1 mg/mL	60
10K	Albumin 1 mg/mL	30
	Albumin 0.5 mg /mL	10
	Albumin 0.1 mg/mL	10
30K	IgG 1 mg/mL	30
	IgG 0.5 mg /mL	30
	IgG 0.1 mg/mL	10
100K	Thyroglubulin 1 mg/mL	30
	Thyroglubulin 0.5 mg /mL	10
	Thyroglubulin 0.1 mg/mL	10

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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01/14 PN 89118C