Microsep™ 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.

Overview

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

Description

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 Supor™ polyethersulfone membrane from Cytiva 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 3000 to 7500 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 14000 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.

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 150 K protein, a 30 K 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 × 680 = 1360000 Daltons = 1360K Daltons; a 100 K Microsep Advance centrifugal device would be the appropriate selection.

The table below is a guide for initial selection of Microsep Advance centrifugal device MWCO 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	Membrane nominal pore size[1]	Biomolecule molecular size	Biomolecule molecular weight	Nucleic acid	
				Base pair (DS)	Bases (SS)
1 K	-	-	3 to 9 K	5 to 16 bp	9 to 32 bs
3 K	-	-	9 to 30 K	16 to 50 bp	32 to 95 bs
10 K	-	-	30 to 90 K	50 to 145 bp	95 to 285 bs
30 K	-	30 to 90 nm	90 to 300 K	145 to 475 bp	285 to 950 bs
100 K[2]	10 nm	90 to 200 nm	300 to 900 K	475 to 1450 bp	950 to 2900 bs

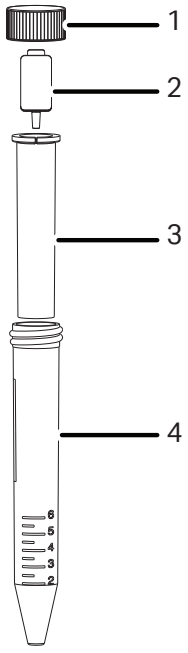
[1] Nominal pore size as measured by electron microscopy

[2] Virus or particle diameter is 30 to 90 nm

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.



Part	Description
1	Sample reservoir cap
2	Encapsulated membrane
3	Sample reservoir
4	Filtrate receiver

Microsep Advance centrifugal device operation

Instructions for use

Step	Action
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 × 100 mm conical-end tubes. ***Note:*** *Always counterbalance the rotor with another Microsep Advance centrifugal device containing the equivalent sample volume.*
3	Spin device at recommended force for required time. • Ultrafiltration: spin at 3000 to 7500 × 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, on page 3* and *Table 3, on page 3* to determine appropriate protocol. • Microfiltration: spin at up to 14000 × 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 sample reservoir: remove and discard the sample reservoir and tightly cap the filtrate receiver for storage.

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 14000 × 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.

Step	Action
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.
	Note: *Use device within 20 minutes to prevent irreversible membrane damage due to dehydration.*

Diafiltration (desalting and buffer exchange)

Step	Action
1	For salt removal or buffer exchange, first concentrate sample at least 10-fold (i.e., 1 mL concentrated to 100 µL).
2	Reconstitute with exchange buffer and reconcentrate 10-fold.
3	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.

Step	Action
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).

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

MWCO	Solute	Time to 25 × concentration (min)		
		3000 × g	5000 × g	7500 × g
3 K	Cytochrome C (0.25 mg/mL)	75	75	75
10 K	Albumin (1 mg/mL)	20	15	10
30 K	IgG (1 mg/mL)	20	15	15
100 K	Thyroglubulin (1 mg/mL)	15	15	10

Table 3. Effects of starting protein concentration

MWCO	Solute	Time to 25 × concentration (min)
3 K	Cytochrome C 1 mg/mL	60
	Cytochrome C 0.5 mg /mL	60
	Cytochrome C 0.1 mg/mL	60
10 K	Albumin 1 mg/mL	30
	Albumin 0.5 mg /mL	10
	Albumin 0.1 mg/mL	10
30 K	IgG 1 mg/mL	30
	IgG 0.5 mg /mL	30
	IgG 0.1 mg/mL	10
100 K	Thyroglubulin 1 mg/mL	30
	Thyroglubulin 0.5 mg /mL	10
	Thyroglubulin 0.1 mg/mL	10

Specifications

Parameter	Specification	
Materials of construction	Filtration media	Ulrtrafiltration: 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°C to 40°C (32 to 104 ºF)	
pH range	Ultrafiltration: 2 to 14 Microfiltration: 1 to 14	
Maximum centrifugal force	Ultrafiltration: 7500 × g Microfiltration: 14000 × g	
Centrifuge	Fits centrifuges that accept standard 17 × 100 mm conical-end tubes	
Sanitization	Provided non-sterile, may be sanitized by filtering 70% ethanol through the device prior to use	

Ordering information

Table 4. Microsep Advance centrifugal devices

Description	24/pkg	100/pkg
3 K Omega	MCP003C41	MCP003C46
10 K Omega	MCP010C41	MCP010C46
30 K Omega	MCP030C41	MCP030C46
100 K Omega	MCP100C41	MCP100C46
0.2 µm Supor	MCPM02C67	MCPM02C68
0.45 µm Supor	MCPM45C67	MCPM45C68

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 L samples.

AcroPrep™ 24 well, AcroPrep Advance 96 well and AcroPrep 384 well filter plates for high throughput concentration, desalting and buffer exchange.

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