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Centriprep® Centrifugal Filter Devices

Centriprep® 3K and 50K devices for research use only; not for use in diagnostic procedures

Introduction

Centriprep® centrifugal filter devices are disposable ultrafiltration devices used for purifying, concentrating, desalting, and filtering biological samples in the 2–15 mL volume range. These ready-to-use ultrafiltration devices are designed for operation in most centrifuges that can accommodate 50 mL centrifuge tubes. They are easy to use and offer a high flow rate. The Centriprep® device consists of a sample container with a twist-lock cap, a filtrate collector containing a low adsorptive Ultracel® regenerated cellulose membrane, and an air-seal cap for sample isolation.

The Centriprep® device design allows filtration and fine particle sedimentation to occur at the same time without clogging the membrane. With a Centriprep® 50K device, a 15 mL sample can be concentrated to 0.6 mL in just 20 minutes and concentration of smaller volumes takes even less time. High concentration factors are achieved because of the membrane's low adsorptivity, and greater than 90% recovery of retained macromolecular solutes is typical. A built-in deadstop provides a final concentrated sample volume of 0.5–0.6 mL.

The Centriprep® product line includes 4 different molecular weight cutoffs (MWCO):

- Centriprep® 3K device — 3,000 MWCO (yellow membrane support)
- Centriprep® 10K device — 10,000 MWCO (green membrane support)
- Centriprep® 30K device — 30,000 MWCO (colorless membrane support)
- Centriprep® 50K device — 50,000 MWCO (pink membrane support)

NOTE: Centriprep® 10K and 30K devices are the only Centriprep® devices intended for in vitro diagnostic use.

Applications

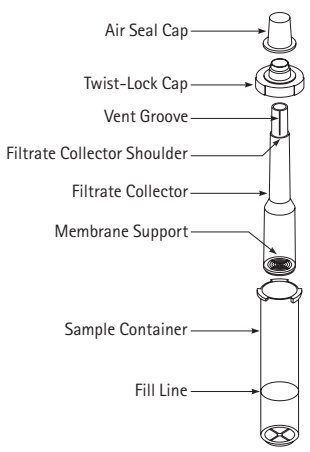
Centriprep® 3K centrifugal filter devices:

- Concentrating and desalting oligonucleotides, peptides, growth factors, and small proteins.

Centriprep® 10K, 30K, and 50K centrifugal filter devices:

- Concentrating and desalting column eluates and gradient fractions containing proteins, enzymes, and antibodies
- Recovering biomolecules from cell culture supernatants, lysates, extracts, or other biological samples
- Purifying low-molecular-weight components (e.g., amino acids and antibiotics) from physiological fluids, cell culture media, or fermentation broths.

Centriprep® Device Components



Limitations

With fixed-angle rotors, polarization control may be adversely affected at low g-forces (below 2,000 × g). Depending on sample composition and solute concentration, filtrate flow may be reduced. When using either type of centrifuge, do not exceed the following limits because excessive g-force may result in leakage or damage to the device.

Device	Maximum Centrifugal Force
Centriprep® 3K	3,000 × g
Centriprep® 50K	1,500 × g

Device Storage and Shelf Life

For best results, store Centriprep® devices at 15–30 °C. Performance may be adversely affected if the devices are stored at extreme temperatures (i.e., below 0 °C or above 40 °C). Shelf life is three years from date of manufacture.

Rinsing Before Use

The membranes in Centriprep® centrifugal filter devices contain trace amounts of glycerine. If this interferes with analysis, fill the device with approximately 15 mL of buffer or deionized water and centrifuge until the first equilibration point is reached. Decant filtrate and concentrate. Repeat as necessary until no more interference is noted. Once wet, membranes must be kept wet to remain functional.

Membrane and Device Appearance

- Occasionally, an unused Centriprep® device may have a few drops of moisture in the filtrate collector or sample container. This is due to condensation of moisture in the membrane and does not affect performance.
- After centrifugation, a small amount of filtrate (approximately 0.5 mL) may remain in the area between the membrane and the membrane support of the Centriprep® device. This is normal and does not affect device operation or performance.
- The Ultracel® membrane used in the Centriprep® centrifugal filter devices may appear slightly wrinkled after spinning.

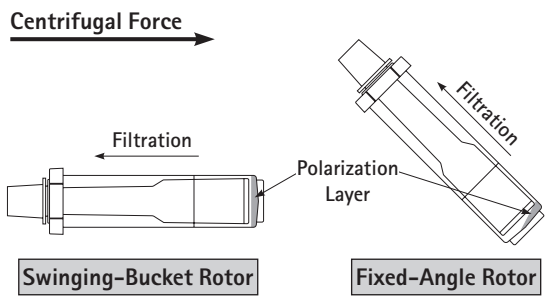
Centrifugal Filter Device Reuse

Centriprep® devices are intended for single use only. Attempts to clean the device may damage the membrane surface or the seal, possibly compromising the unit's integrity and thereby affecting performance.

Principle of Operation

To begin operation, the sample container's twist-lock cap is loosened and the filtrate collector is removed. Sample is added, then the filtrate collector is carefully reinserted back into the sample container (displacing solution) and the cap locked, sealing the device. The assembled centrifugal filter device is centrifuged at 1,000–3,000 × g, depending on the membrane MWCO used.

Immersing the filtrate collector in the sample solution creates a slight hydrostatic pressure differential that exerts an upward buoyancy force on the membrane at the filtrate collector bottom. By itself, the hydrostatic pressure exerted by the displaced solution is too weak to produce ultrafiltration. Centrifugation increases this pressure, forcing low molecular weight materials and solvent through the membrane into the filtrate collector. Solutes with molecular weights above the membrane MWCO remain in the sample container and become increasingly concentrated as the operation continues. Ultrafiltration occurs in the direction opposite the centrifugal force vector.

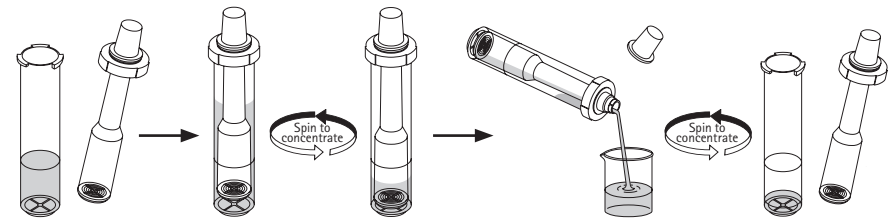


The pressure differential created by the raised solution level is important in centrifugal filter device operation. During centrifugation, the sample solution meniscus falls as the filtrate meniscus rises. In the process, the filtrate collector loses its buoyancy and sinks to the bottom of the sample container, permitting maximum filtrate collection. Eventually, an equilibrium is reached where the menisci are at equal heights. Filtration then stops, since the hydrostatic pressure difference is now zero.

If further concentration is required, decant the filtrate before spinning the device for a second time. Decanting re-establishes the pressure differential between the sample meniscus and the filtrate meniscus, allowing filtration to resume. Filtration continues until a new equilibrium point is achieved. Note that the pressure differential is constantly changing during operation as the menisci rise and fall.

Centriprep® centrifugal filter devices are designed to maintain high flow rates by minimizing solute build-up on the membrane during operation. Centrifugal force causes dense materials to sink away from the membrane to the sample container bottom. Because the Centriprep® membrane "floats" above this polarization layer, the full surface of the membrane is used at all times, and filtrate flow is unrestricted. This is particularly beneficial when working with suspensions, which can easily clog or foul a membrane.

How to Use Centriprep® Centrifugal Filter Devices



Disassemble device and add sample to fill line. | Reassemble and place in centrifuge. | Spin until solution levels have equalized. | Orient vent groove up and decant filtrate. Spin again. | Disassemble and recover concentrate.

1. Turn the twist-lock cap on the sample container counterclockwise, then slide the filtrate collector/cap assembly out and set it aside.

   CAUTION: Take care not to touch, scratch, or damage the membrane on the bottom of the filtrate collector during disassembly, filling, and re-assembly of the device.

2. Add solution to the sample container. The line on the side of the container marks the maximum fill volume of 15 mL. Do not exceed this volume.

   NOTE: For solutions with particulate material, such as cell suspensions, a starting volume over 5 mL will result in reduced flow rates. For best results when working with a sample having over 10% solids, limit the initial sample volume to 5 mL or less.

3. Seat the twist-lock cap fully onto the shoulder of filtrate collector. If necessary, slide the cap downward until it stops at the shoulder.
4. Carefully insert the capped filtrate collector into the sample container, gently pushing down so the collector displaces the solution. Turn the twist-lock cap clockwise to seal the sample container. Finally, make sure the air-seal cap is snug on the twist-lock cap.
5. Insert the assembled Centriprep® device into the centrifuge and counterbalance with a similar device.

   CAUTION: Inspect swinging-bucket rotors for proper clearance before centrifuging. Any obstruction may result in damage to the Centriprep® device and possible loss of sample. For added clearance, the Centriprep® device may be used in adaptors without rubber cushions.

6. Spin the Centriprep® device at the appropriate g-force until the fluid levels inside and outside the filtrate collector equilibrate. For concentration applications, see the guidelines for achieving various concentration volumes outlined in the following "Concentration Times for Dilute Protein Solutions" section.
7. If further concentration is required after equilibration, remove the device from the centrifuge and take off the air-seal cap. With the vent groove oriented upward, decant the filtrate; replace the cap and spin the device a second time. For filtration applications, reserve the filtrate.
8. After the second spin, decant the remaining filtrate. If further concentration is desired, spin the device again; otherwise, proceed to step 9.
9. Loosen the twist-lock cap (turn counterclockwise) and remove the filtrate collector. Withdraw the sample using a pipette, or pour the concentrate into a suitable container.

Typical Concentration Times for Dilute Protein Solutions

Starting Volume in Device	Spin Time (min) and Concentrate Volume (mL)					
Centriprep® 3K	**First Spin**		**Second Spin**		**Third Spin**	
	min	**mL**	**min**	**mL**	**min**	**mL**
5 mL	50	1.2	10	0.8	10	0.5
10 mL	65	2.0	10	1.0	10	0.5
15 mL	95	3.1	35	1.2	10	0.6
Centriprep® 10K	**First Spin**		**Second Spin**		**Third Spin**	
	min	**mL**	**min**	**mL**	**min**	**mL**
5 mL	15	1.3	10	0.6	N/A	
10 mL	30	2.1	10	1.0	5	0.5
15 mL	40	3.2	10	1.2	5	0.5
Centriprep® 30K	**First Spin**		**Second Spin**		**Third Spin**	
	min	**mL**	**min**	**mL**	**min**	**mL**
5 mL	5	1.3	5	0.8	5	0.5
10 mL	10	2.4	5	1.2	5	0.4
15 mL	15	3.2	10	1.1	5	0.5
Centriprep® 50K	**First Spin**		**Second Spin**		**Third Spin**	
	min	**mL**	**min**	**mL**	**min**	**mL**
5 mL	5	1.4	5	1.0	5	0.6
10 mL	10	2.2	5	1.1	5	0.6
15 mL	10	3.4	5	1.3	5	0.6

Spin Conditions: Swinging-bucket rotor, 3,000 × g for 3K and 10K, and 1,500 for 30K and 50K, 25 °C
Protein marker used: Cytochrome c (0.25 mg/mL) for 3K, and bovine serum albumin (1 mg/mL) for 10K, 30K, and 50K.
N/A = not applicable, third spin unnecessary.

Performance

Flow rates and recovery characteristics of Centriprep® centrifugal filter devices have been tested with several well-known solutes. These results, which are summarized in the following sections, may be used to estimate performance with other solutes.

Performance, continued

Relative centrifugal force (RCF) is measured at the filtrate collector base and is calculated as follows:

$RCF = 1.118 \times 10^{-5} \times \text{radius} \times (\text{RPM})^2$

Radius = distance in centimeters, measured from the center of rotation to the base of the filtrate collector

RPM = revolutions per minute.

NOTE: RCF and RPM are not the same (i.e., 3,000 × g is NOT equivalent to 3,000 RPM). Check the centrifuge operating guide for instructions on converting g-force to RPM, or use the formula above.

Flow Rate

Filtration rate is affected by several operating parameters, including sample concentration, starting volume, relative centrifugal force, type of rotor used, membrane MWCO, and temperature. When concentrating dilute solutions, swinging-bucket and fixed-angle rotors yield comparable flow rates.

For solutions containing over 10% solids, a starting volume greater than 5 mL typically results in reduced flow rates. A volume of 5 mL or less produces the fastest flow rates because this limits the amount of solids that can pack onto and blind the membrane.

Spin times must be lengthened when working at low temperatures. At 4 °C, flow rates are approximately 1.5 times slower than at 25 °C. Viscous solutions may also require longer spin times. For example, a 1 mg/mL BSA solution in 50% glycerine would take five times longer to concentrate than the identical protein in an aqueous buffer solution, using a Centriprep® 10K device.

Retention and Recovery

The anisotropic, hydrophilic membranes in Centriprep® centrifugal filter devices are characterized by a molecular weight cutoff; i.e., their ability to reject molecules above a specified molecular weight.

Because it is an easy parameter to work with, molecular weight is used in rating membrane permeability and selectivity based on results achieved with well-characterized proteins.

However, molecular weight may not be valid for all solutes. For instance, retentivity may be greater for a globular molecule than for a linear molecule having the same nominal molecular weight. The effects of pH and ionic strength on a molecule's dimensions and behavior in a particular solution must also be considered when assessing solute retentivity.

Low solute recovery in the concentrate may indicate possible adsorptive losses and/or solute passage through the membrane. Adsorptive losses depend on solute concentration, the nature of solute (hydrophobic or hydrophilic), temperature, time of contact with component surfaces, sample composition, and pH.

Typical Concentrate Recovery

Solute & Starting Concentration	Nominal Molecular Weight	Centriprep® % Concentrate Recovery 3K	10K	30K	50K
IgG Fr. II (1 mg/mL)	156,000	–	–	–	99
Bovine serum albumin (1 mg/mL)	67,000	–	97	98	93
Ovalbumin (1 mg/mL)	45,000	–	95	90	–
α-Chymotrypsinogen (1 mg/mL)	25,000	94	93	–	–
Cytochrome c (0.25 mg/mL)	12,400	96	96	8	4
Vitamin B12 (0.2 mg/mL)	1,355	12	–	5	–
15 mL starting volume, 25 °C, swinging-bucket rotor					
Results based on recommended spin times outlined in the "Concentration Times for Dilute Protein Solutions" section					

Typical Protein Recovery, Centriprep® 30K Device

Concentration	% Concentrate Recovery ± 1 SD
100 µg/mL	98.2 ± 1.7
1,000 µg/mL	96.6 ± 0.2
15 mL starting volume, 25 °C, swinging-bucket rotor, 1,500 × g Bovine serum albumin, two 25-minute spins	

Desalting

For desalting or solvent exchange, the sample is concentrated until the equilibration point is reached. Filtrate is discarded, then the sample is reconstituted to the original volume by adding an appropriate solvent and vortex mixing. The sample is then concentrated and reconstituted once more. This process is repeated until the concentration of the contaminating microsolute is sufficiently reduced.

The following theoretical example illustrates the process of sample desalting:

Process Step	Sample Volume	Protein Concentration	NaCl Concentration
Initial sample	15 mL	1 mg/mL	500 mM
Spin to concentrate	3 mL	5 mg/mL	500 mM
Reconstitute back to 15 mL with 10 mM NaCl	15 mL	1 mg/mL	108 mM
Spin to concentrate/ desalt #1	3 mL	5 mg/mL	108 mM
Reconstitute back to 15 mL with 10 mM NaCl	15 mL	1 mg/mL	29.6 mM
Spin to concentrate/ desalt #2	3 mL	5 mg/mL	29.6 mM
Reconstitute back to 15 mL with 10 mM NaCl	15 mL	1 mg/mL	13.9 mM
Spin to concentrate/ desalt #3	3 mL	5 mg/mL	13.9 mM

Performance Figures

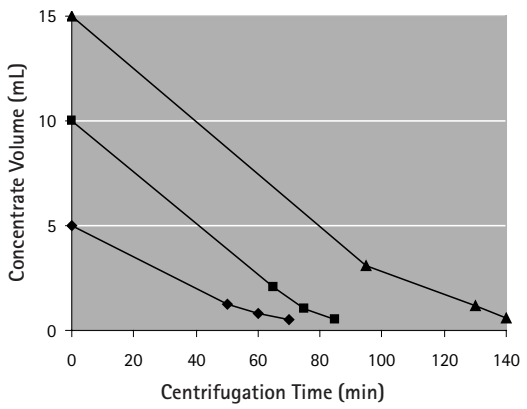
Typical Filtration Profiles for Centriprep® Devices Using Common Dilute Protein Solutions

Centriprep® 3K Device

Operating Parameters:
- g-force: 3,000 × g
- temperature: 25 °C
- swinging-bucket rotor
- solute: 0.25 mg/mL Cytochrome c

5, 10, and 15 mL starting volumes

Filtrate decanted at times indicated

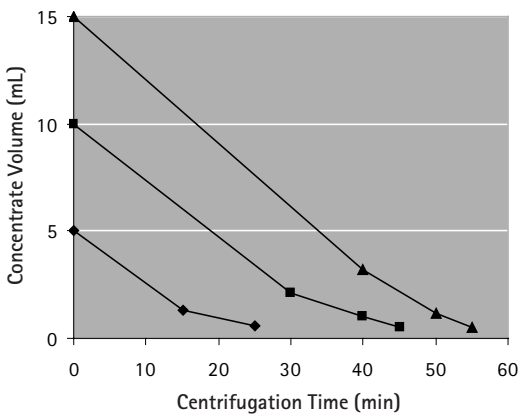


Centriprep® 10K Device

Operating Parameters:
- g-force: 3,000 × g
- temperature: 25 °C
- swinging-bucket rotor
- solute: 1 mg/mL Bovine serum albumin

5, 10, and 15 mL starting volumes

Filtrate decanted at times indicated

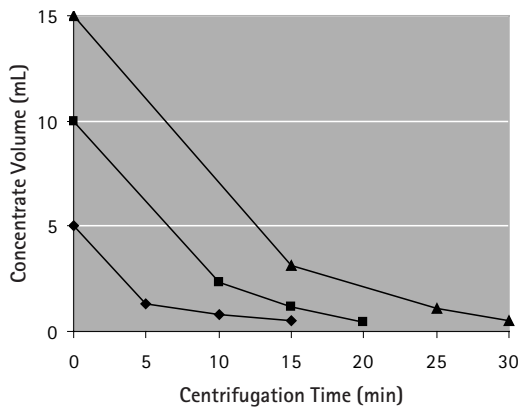


Centriprep® 30K Device

Operating Parameters:
- g-force: 1,500 × g
- temperature: 25 °C
- swinging-bucket rotor
- solute: 1 mg/mL Bovine serum albumin

5, 10, and 15 mL starting volumes

Filtrate decanted at times indicated

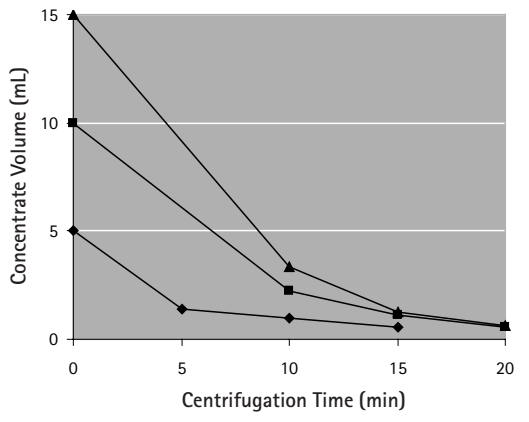


Centriprep® 50K Device

Operating Parameters:
- g-force: 1,500 × g
- temperature: 25 °C
- swinging-bucket rotor
- solute: 1 mg/mL Bovine serum albumin

5, 10, and 15 mL starting volumes

Filtrate decanted at times indicated



How to Quantify Recoveries

Calculate total recovery, percent concentrate recovery, and percent filtrate recovery using the method below. This procedure provides a close approximation of recoveries for solutions having concentrations up to roughly 20 mg/mL.

NOTE: Appropriate assay techniques include absorption spectrophotometry, radioimmunoassay, refractive index, and conductivity.

Weighing Procedure

The density of most dilute proteins is nearly equal to the density of water (i.e., 1 g/mL). Using this property, the concentrate and filtrate volumes can be quantified by weighing them and converting the units from grams to milliliters. This technique is valid only for solutions with concentrations of approximately 20 mg/mL or less.

1. Before use, weigh the sample container and an empty test tube (15 mL capacity).
2. Fill the sample container with solution and reweigh.
3. Assemble the centrifugal filter device and spin per instructions. Decant filtrate into preweighed test tube. If further concentration is desired, spin a second time, decanting filtrate into test tube containing first filtrate. Reweigh the test tube plus filtrate(s).
4. Disassemble the device and reweigh the sample container plus concentrate.
5. Subtract weight of empty test tube or sample container to calculate the weights of the starting material, filtrate, and concentrate.
6. Assay the starting material, filtrate, and concentrate to determine solute concentration.
7. Calculate recoveries using the weight/volume data and the measured concentrations as follows:

$$\%\ \text{concentrate recovery} = 100 \times \frac{W_c \times C_c}{W_o \times C_o}$$

$$\%\ \text{filtrate recovery} = 100 \times \frac{W_f \times C_f}{W_o \times C_o}$$

$$\%\ \text{total recovery} = \%\ \text{concentrate recovery} + \%\ \text{filtrate recovery}$$

W_c = total weight of concentrate before assay
W_o = weight of original starting material
W_f = weight of filtrate
C_c = concentrate concentration
C_o = original starting material concentration
C_f = filtrate concentration

Chemical Compatibility

Centriprep® devices are intended for use with biological fluids and aqueous solutions. Before use, check the sample for chemical compatibility with the device. The solutions listed in the following table have been evaluated for chemical compatibility with Centriprep® devices.

Acids	Concentration		Concentration
Acetic acid	≤ 80%	Phosphoric acid	≤ 30%
Formic acid	≤ 50%	Sulfuric acid	≤ 3%
Hydrochloric acid	≤ 1.0 N	Trichloroacetic acid (TCA)	≤ 10%
Lactic acid	≤ 50%	Trifluoroacetic acid (TFA)	≤ 50%
Nitric acid	≤ 10%		
Alkalis			
Ammonium hydroxide	≤ 5%	Sodium hydroxide	≤ 0.1 N
Alcohols			
n-Butanol	≤ 90%	Isopropanol	≤ 70%
Ethanol	≤ 70%	Methanol	≤ 60%
Detergents			
Alconox® detergent	≤ 1%	Sodium deoxycholate	≤ 5%
Lubrol® PX detergent	≤ 0.1%	Tergazyme® detergent	≤ 1%
Nonidet™ P-40 surfactant	≤ 2%	Triton® X-100 surfactant	≤ 0.002%
PyroCLEAN™ detergent	1:10	Tween® 20 surfactant	≤ 0.1%
Sodium dodecyl sulfate (SDS)	≤ 2%		
Organic solvents			
Acetone	not recommended	Dioxane	not recommended
Acetonitrile	≤ 30%	Ethyl acetate	not recommended
Benzene	not recommended	Formaldehyde	≤ 5%
Carbon tetrachloride	not recommended	Pyridine	not recommended
Chloroform	not recommended	Tetrahydrofuran	not recommended
Dimethyl formamide	≤ 50%	Toluene	not recommended
Dimethyl sulfoxide (DMSO)	≤ 5%		
Miscellaneous			
Ammonium sulfate	Saturated	Phenol	≤ 1%
Diethyl pyrocarbonate	≤ 0.2%	Phosphate buffer (pH 8.2)	≤ 1 M
Dithiothreitol (DTT)	≤ 0.1 M	Polyethylene glycol	≤ 10%
Glycerine	≤ 70%	Sodium carbonate	≤ 20%
Guanidine HCl	≤ 6 M	Sodium thiocyanate	≤ 3 M
Guanidine thiocyanate	≤ 0.5 M	Tris buffer (pH 8.2)	≤ 1 M
Imidazole	≤ 100 mM	Urea	≤ 8 M
Mercaptoethanol	≤ 0.1 M		

Specifications

Sample container capacity	15 mL
Final concentrate volume at 25 °C	0.5–0.6 mL
Filtrate collector capacity	12 mL
Maximum relative centrifugal force	
Centriprep® 3K device	3,000 × g
Centriprep® 50K device	1,500 × g
Active membrane area	2.84 cm^2
Hold-up volume (membrane and support)	0.5 mL
Dimensions	
Diameter of twist-lock cap	36.5 mm (cap resides outside centrifuge carrier)
Diameter of sample container	28.1 mm
Length	135 mm
Materials of Construction	
Membrane	Ultracel® regenerated cellulose
Sample container	Polycarbonate
Membrane support	Polycarbonate
Filtrate collector	Polycarbonate
Twist-lock cap	Nylon
Air seal cap	Polypropylene

Product Ordering Information

This section lists the catalogue numbers for Centriprep® Ultrafiltration Devices.

Description	24/pk	96/pk
For in vitro diagnostic use		
Centriprep® 10K device (10,000 MWCO)	4304	4305
Centriprep® 30K device (30,000 MWCO)	4306	4307
For research use only		
Centriprep® 3K device (3,000 MWCO)	4302	4303
Centriprep® 50K device (50,000 MWCO)	4310	4311

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