NeoSpin Centrifugal Filters

NB-57-0001-1
NB-57-0001-2
NB-57-0002-1
NB-57-0002-2
NB-57-0004-1
NB-57-0004-2
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NB-57-0011
NB-57-0012
NB-57-0013
NB-57-0014
NB-57-0015

Contents

Neo Biotech
74 rue des Suisses – 92000 Nanterre

NeoSpin centrifugal filter

#Cat: NB-57-0001-1 Size: 8pcs
#Cat: NB-57-0001-2 Size: 24pcs
#Cat: NB-57-0002-1 Size: 8pcs
#Cat: NB-57-0002-2 Size: 24pcs
#Cat: NB-57-0004-1 Size: 8pcs
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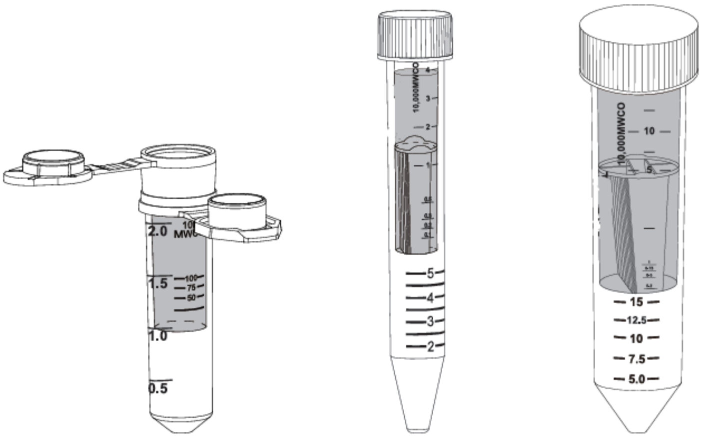


Introduction

Centrifugal filters are disposable, single-use only ultrafiltration devices with polyethersulfone (PES) membranes for the centrifugal concentration and/or purification of biological samples. This guide will help you choose the best centrifugal filters for your application.

Major Uses for Ultrafiltration

Ultrafiltration is a convective process that uses anisotropic semi-permeable membranes to separate macromolecular species and solvents primarily on the basis of size. It is particularly appropriate for the concentration of macromolecules and can also be used to purify molecular species or for solvent exchange (Table 1). Ultrafiltration is a gentle, non-denaturing method that is more efficient and flexible than alternative processes.

Solute Concentration

Ultrafiltration membranes are used to increase the solute concentration of a desired biological species. The filtrate is cleared of macromolecules which are significantly larger than the retentive membrane pores. Microsolute is removed convectively with the solvent.

Solute Desalting or Purification

A solution may be purified from salts, non-aqueous solvents, and generally from low molecular weight materials. Multiple solvent exchanges will progressively purify macromolecules from contaminating solutes. Microsolutes are removed most efficiently by adding solvent to the solution being ultrafiltered at a rate equal to the speed of filtration. This is called diafiltration.

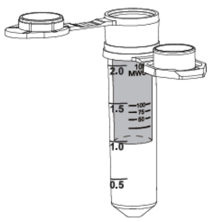
Table 1. Typical Ultrafiltration Applications

1). General purpose laboratory concentration and desalting of proteins, enzymes, cells, biomolecules, antibodies, and immunoglobulins
2). Removal of labeled amino acids and nucleotides
3). HPLC sample preparation
4). Deproteinization of samples
5). Recovery of biomolecules from cell culture supernatants, lysates

Choosing the Right Centrifugal Filters

NeoSpin offers NeoSpin Centrifugal Filters in three sizes. The information below and Tables 2 and 3 will help you find the best centrifugal filters for your needs.

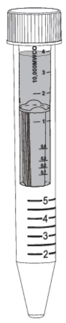
1. **NeoSpin 0.5mL Centrifugal Filters for 0.1 to 0.5 mL samples**



Centrifugal filters offer a simple, one-step procedure for samples preparation. They can effectively be used in fixed angle rotors accepting 2 mL centrifuge tubes.

The vertical membrane design and thin channel filtration chamber minimizes membrane fouling and provides high speed concentrations, even with particle laden solutions.

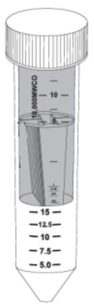
2. **NeoSpin 5 mL Centrifugal Filters for 2 to 5 mL samples**



Centrifugal filters offer increased volume flexibility and performance. Centrifugal filters can process up to 5 mL in swing bucket rotors accepting standard 15 mL conical bottom tubes. In a single spin, solutions can be concentrated in excess of 100-fold. Samples are typically concentrated in 10 to 30 minutes with macromolecular recoveries in excess of 95%.

The features twin vertical membranes for unparalleled filtration speeds and 100X plus concentrations. Remaining volume is easy to read off the printed scale on the side of the centrifugal filters, and the modified dead stop pocket further simplifies direct pipet recovery of the final concentrate

3. **NeoSpin 15 mL Centrifugal Filters for 5 to 15 mL samples**



Centrifugal filters offer increased volume flexibility and performance. Centrifugal filters handle up to 15 mL in swing bucket centrifuges and 12 mL in 25° fixed angle rotors accepting 50 mL centrifuges tubes.

Featuring twin vertical membranes for unparalleled filtration speeds, the centrifugal filters can achieve 100X plus concentrations. The remaining volume is easy to read off the printed scale on the side of the centrifugal filters, and the modified dead stop pocket further simplifies direct pipet recovery of the final concentrate.

Table 2. NeoSpin Centrifugal Filters Performance Characteristics

Centrifugal Filters	**0.5mL**	**5mL**	**15mL**
Capacity			
Swing bucket rotor	Do not use	5mL	15mL
Fixed angle rotor	0.5mL	4mL	12mL
Minimum rotor angle	40°	25°	25°

Dimensions

Total Length	48.1mm	123.4mm	119.5mm
Width	12.9mm	22mm	33.7mm
Active membrane area	0.65cm^2	3.5cm^2	9.7cm^2
Membrane hold up volume	<5µl	<10µl	<20µl
Dead stop volume*	20 µl	30 µl	50 µl

Materials of Construction

Body	PP	PP	PP
Filtrate vessel	MBS	MBS	MBS
Centrifugal filters cap	PP	HDPE	HDPE
Membrane	PES	PES	PES

*Dead stop volume as designed in molding tool. This volume may vary depending on sample, sample concentration, operation temperature, and centrifuge rotor.

Choosing The Best Molecular Weight Cut-off (MWCO) Membrane

Centrifugal filters use general purpose polyethersulfone membranes that provide excellent performance with most solutions when retentate recovery is of primary importance. Polyethersulfone membranes exhibit no hydrophobic or hydrophilic interactions and are usually preferred for their low fouling characteristics, exceptional flux, and broad pH range.

Table 3. PES Membrane Selection Guide (recommended MWCO*)

Application	<5,000	10,000	30,000	50,000	100,000
Bacteria					●
Enzymes	●	●			
Growth factors	●	●			
Immunoglobulins			●	●	●
MAB			●	●	●
Peptides	●		●	●	
Virus			●	●	●
Yeast					●

*For highest recovery, select a membrane MWCO which is at least half of the molecular weight of the solute to be retained.

The advanced designs and low adsorption materials that characterize NeoSpin products offer a unique combination of faster processing speeds and higher recovery of the concentrated sample. Providing that the appropriate device size (Table 2) and membrane cut-off (Table 3) are selected, products will typically yield recoveries of the concentrated sample in excess of 80% when the starting sample contains over 0.1 mg/mL of the solute of interest (Table 4). Most of the loss is caused by nonspecific binding both to the membrane surface and to exposed binding sites on the plastic of the sample container.

Table 4. NeoSpin Centrifugal Filters Performance Characteristics

Spin condition: for NeoSpin 0.5mL/5mL/15mL, fixed angle rotor of 10,000g/5,000g/4,000g and swing bucket rotor of 4,000g/3,000g for centrifugation, room temperature, n=6/4/2.

Centrifugal Filters	0.5mL		5mL				15mL			
Rotor	40° Fixed Angle		Swing Bucket		25° Fixed Angle		Swing Bucket		25° Fixed Angle	
Start volume	0.5mL		5mL		4mL		15mL		12ml	
	Min.	Rec.	Min.	Rec.	Min.	Rec.	Min.	Rec.	Min.	Rec.
Cytochrome C (0.25mg/mL)										
5,000 MWCO PES	15	91%	45	92%	45	94%	30	86%	30	94%
BSA 1.0mg/ml (66,000 MW)										
10,000 MCWO PES	5	87%	20	95%	20	95%	30	86%	30	85%
30,000 MWCO PES	5	92%	10	99%	10	88%	20	98%	20	98%
IgG 0.5mg/mL (160,000 MW)										
50,000 MCWO PES	5	96%	10	96%	10	96%	15	96%	15	96%
100,000 MWCO PES	5	84%	10	82%	10	94%	20	84%	20	86%

Adsorption to the Membrane

Depending on sample characteristics relative to the membrane type used, solute adsorption on the membrane surface is typically 2 to 10 µg/cm^{2}. This can increase to 20 to 100 µg/cm^{2} when the filtrate is of interest and the solute must pass through the whole internal structure of the membrane. Typically, a higher cut-off membrane will bind more than a low molecular weight cut-off membrane.

Adsorption to the Sample Container

Although every effort is made to minimize this phenomenon by the selection of low adsorption materials and tool production to optical standards, some solute will blind to the internal surface of the sample container. While the relative adsorption will be proportionately less important on the sample container than on the membrane, due to the higher total surface area, this can be the major source of yield loss.

Protein Retention

The membranes used in NeoSpin Centrifugal Filters devices are characterized by a molecular weight cutoff (MWCO); that is, their ability to retain molecules above a specified molecular weight. Solutes with molecular weights close to the MWCO may be only partially retained. Membrane retention depends on the solute’s molecular size and shape. For most applications, molecular weight is a convenient parameter to use in assessing retention characteristics. For best results, use a membrane with a MWCO at least two times smaller than the molecular weight of the protein solute that one intends to concentrate.
Refer to Table 5, Table 5, and Table 7.

Table 5. Typical Retention of Protein Markers 0.5mL
(Spin condition:10000g, room temperature, 0.5mL starting volume. Protein markers used: Cytochrome c for 5K, BSA for 10K,30K and 50K, and IgG for 100K, n=6)

Rotor	**40° Fixed Angle**	
Start Volume	**0.5mL**	
	Min.	**Rec.**
Cytochrome C (0.25 mg/mL) 5,000 MWCO PES	15	≥95%
BSA 1.0 mg/mL (66,000 MW) 10,000 MWCO PES	5	≥95%
30,000 MWCO PES	5	≥95%
IgG 0.5 mg/mL (160,000 MW) 50,000 MWCO PES	5	≥80%
100,000 MWCO PES	5	≥90%

Table 6. Typical Retention of Protein Markers 5mL

(Spin condition: Swing bucket rotor, 4000g, or fixed angle rotor, 5000g for 5K, 10K,30K,50K and 100K, room temperature,5mL or 4mL starting volume. n=4)

Rotor		**Swing Bucket**	**25°Fixed Angle**
Start Volume		**5 mL**	**4 mL**
	Min.	**Rec.**	**Rec.**
Cytochrome C (0.25 mg/mL)			
5,000 MWCO PES	45	≥90%	≥90%
BSA 1.0 mg/mL (66,000 MW)			
10,000 MWCO PES	20	≥95%	≥95%
30,000 MWCO PES	10	≥95%	≥95%
IgG 0.5 mg/mL (160,000 MW)	10		
50,000 MWCO PES		≥80%	≥80%
100,000 MWCO PES	10	≥80%	≥95%

Table 7. Typical Retention of Protein Markers 15mL

Rotor		**Swing Bucket**	**25° Fixed Angle**
Start Volume		**15 mL**	**12 mL**
	Min.	**Rec.**	**Rec.**
Cytochrome C (0.25 mg/mL)			
5,000 MWCO PES	30	≥80%	≥90%
BSA 1.0 mg/mL (66,000 MW)			
10,000 MWCO PES	30	≥95%	≥95%
30,000 MWCO PES	20	≥95%	≥95%
IgG 0.5 mg/mL (160,000 MW)			
50,000 MWCO PES	15	≥80%	≥80%
100,000 MWCO PES	20	≥90%	≥90%

Operation

1.Select the most appropriate membrane cut-off for your sample. For maximum recovery select a molecular weight cut off (MWCO) at least 50% smaller than the molecular size of the species of interest.

2.Fill centrifugal filters with up to maximum volumes shown in Table 4 (page 5). Ensure screw closure is fully seated.

3.Insert assembled centrifugal filters into centrifuge (when fixed angle rotors are used, angle concentrator so that the printed window faces upwards/out-wards).

4.Centrifuge at speeds recommended in Table 8 (page9), taking care not to exceed the maximum g force indicated by membrane type and MWCO.

5.Once the desired concentration is achieved, (see Tables 4,5,6, and7, page5,6 and7) for guide to concentration times, remove assembly and recover sample from the bottom of the concentrate pocket with a pipet.

Desalting/Buffer Exchange

1.Concentrate sample to desired level.

2.Empty filtrate container.

3.Refill centrifugal filters with an appropriate solvent.

4.Concentrate the sample again and repeat the process until the concentration of the contaminating microsolute is sufficiently reduced. Typically,3 wash cycles will remove 99% of initial salt content.

Helpful Hints

Flow Rate

Flow rate is affected by several parameters, including MWCO, porosity, sample concentration, viscosity, centrifugal force, and temperature. Expect significantly longer spin times for starting solutions with over 5% solids. When operating at 4°C, flow rates are approximately 1.5 times slower than at 25°C. Viscous solutions such as 50% glycerin will take up to 5 times longer to concentrate than samples in a predominantly buffer solution.

Prerinsing

Membranes fitted to centrifugal filters contain trace amounts of glycerin and sodium azide. Should these interfere with analysis, they can be removed by rinsing fill volume of buffer solution or deionized water through the centrifugal filters. Decant filtrate and concentrate before processing sample solution. If you do not want to use the prerinsed device immediately, store it in the refrigerator with buffer or water covering the membrane surface. Do not allow the membrane to dry out.

Sterilization of Polyethersulfone Membranes

Polyethersulfone membranes should not be autoclaved as high temperatures will substantially increase membrane MWCO.To sanitize or sterilize these devices, use a 70% ethanol solution or sterilizing gas mixture.

Optimizing Solute Recovery

When highest solute recoveries are most important, in particular when working with solute quantities in the microgram range, recommends considering the following key points:

- Select the smallest device that suits the sample volume. Additionally, take advantage of the extra speed of centrifugal filters by refilling a smaller centrifugal filter repeatedly.
- Select the lowest MWCO membrane that suits the application.
- When available, use swing bucket rotors rather than fixed angle rotors. This reduces the surface area of the centrifugal filter that will be exposed to the solution during centrifugation.
- Reduce centrifugal force to approximately half of the maximum recommended (Table 8).
- Avoid over-concentration. The smaller the final concentrate volume, the more difficult it is to achieve complete recovery. If feasible, after a first recovery, rinse the device with one or more drops of buffer and then recover again.

Table 8. Maximum Recommended Centrifugal Force

Centrifugal Filters	0.5mL	5mL	15mL
Maximum Spin Force-Swing Bucket			
5,000 to 100,000 MWCO PES	Do not use	4,000 xg	3,000 xg
Maximum Spin Force-Fixed Angle			
5,000 to 100,000 MWCO PES	10,000 xg	5,000 xg	4,000 xg

Chemical Compatibility

Centrifugal filters are designed for use with biological fluids and aqueous solutions. For chemical computability details, refer to Table 9.

Table 9. Chemical Compatibility*

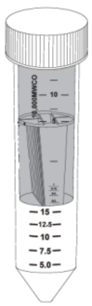
(2-hour contact time; compatible pH range, pH 1-9)

Acetic Acid (25.0%)	1	Lactic Acid (5.0%)	1
Acetone (10.0%)	3	Mercaptoethanol (10mL)	1
Acetonitrile (10.0%)	3	Methanol (60%)	2
Ammonium Hydroxide (5.0%)	2	Nitric Acid (10.0%)	1
Ammonium Sulphate (saturated)	1	Phenol (1.0%)	2
Benzene (100%)	3	Phosphate Buffer (1.0M)	1
n-Butanol (70%)	1	Polyethylene Glycol (10%)	1
Chloroform (1.0%)	3	Pyridine (100%)	2
Dimethyl Formamide (10.0%)	2	Sodium Carbonate (20%)	2
Dimethyl Sulfoxide (5.0%)	1	Sodium Deoxycholate (5.0%)	1
Ethanol (70.0%)	1	Sodium Dodecylsulfate (0.1M)	1
Ethyl Acetate (100%)	3	Sodium Hydroxide	3
Formaldehyde (30%)	1	Sodium Hypochlorite (200ppm)	2
Formic Acid (5.0%)	1	Sodium Nitrate (1.0%)	1
Glycerine (70%)	1	Sulfamic Acid (5.0%)	1
Guanidine HCL (6M)	1	Tetrahydrofuran (5.0%)	3
Hydrocarbons, aromatic	3	Toluene (1.0%)	3
Hydrocarbons, chlorinated	3	Trifluoroacetic Acid (10%)	1
Hydrochloric Acid (1M)	1	Tween 20 (0.1%)	1
Imidazole (500mM)	1	Triton X-100 (0.1%)	1
Isopropanol (70%)	1	Urea (8M)	1

*1=acceptable;2=questionable, testing advised;3=not recommended

Ordering Information

NeoSpin Centrifugal Filters



Cat. No.	Capacity	Membrane	Qty/Box	Qty/Cs
NB-57-0011	0.5mL	5,000 MWCO	25	300
NB-57-0012	0.5mL	10,000 MWCO	25	300
NB-57-0013	0.5mL	30,000 MWCO	25	300
NB-57-0014	0.5mL	50,000 MWCO	25	300
NB-57-0015	0.5mL	100,000 MWCO	25	300
NB-57-0006	5mL	5,000 MWCO	24	96
NB-57-0007	5mL	10,000 MWCO	24	96
NB-57-0008	5mL	30,000 MWCO	24	96
NB-57-0009	5mL	50,000 MWCO	24	96
NB-57-0010	5mL	100,000 MWCO	24	96
NB-57-0001-1	15mL	5,000 MWCO	8	96
NB-57-0001-2	15mL	5,000 MWCO	24	96
NB-57-0002-1	15mL	10,000 MWCO	8	96
NB-57-0002-2	15mL	10,000 MWCO	24	96
NB-57-0003-1	15mL	30,000 MWCO	8	96
NB-57-0003-2	15mL	30,000 MWCO	24	96
NB-57-0004-1	15mL	50,000 MWCO	8	96
NB-57-0004-2	15mL	50,000 MWCO	24	96
NB-57-0005-1	15mL	100,000 MWCO	8	96
NB-57-0005-2	15mL	100,000 MWCO	24	96

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