Nanosep™ centrifugal devices

For simple, reliable concentrating and desalting of 50 to 500 µL in minutes

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.

Background

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

Nanosep™ centrifugal devices provide rapid and convenient concentration, fractionation, and salt removal of 50 to 500 µL biological samples. A starting sample volume of 500 µL can be concentrated 100-fold to 5 µL within 4 to 20 minutes. The Nanosep devices' ease of use saves valuable time.

Each Nanosep centrifugal device is constructed of polypropylene and contains low protein-binding Omega™ membrane that provides reduced non-specific adsorption and high recoveries. Nanosep devices are compatible with a number of solvents, allowing them to be used in a variety of different applications. The devices are ideal for concentrating proteins, oligonucleotides, nucleic acids, enzymes, and antibodies.

Principle of Nanosep centrifugal devices

Centrifugation up to 14 000 × g (5000 × g for DNA) provides the driving force for filtration, moving sample toward the encapsulated Omega membrane. Biomolecules larger than the nominal molecular weight cutoff of the membrane are retained in the sample reservoir. Solvent and low molecular weight molecules pass through the membrane into the filtrate receiver.

Molecular weight cutoffs available

Nanosep centrifugal devices are available with five different molecular weight cutoffs (MWCO): 3K, 10K, 30K, 100K, and 300K.

Applications

Nanosep centrifugal devices replace dialysis, chemical precipitation, and lyophilization in the following applications:

- Concentration of dilute samples
- Buffer exchange or salt removal of chromatography eluates or gradient fractions
- Recovery of growth factors from cell culture supernatants or cell lysates
- Separation of PCR products from primers
- Removal of unincorporated nucleotides from hybridization probes
- Concentration and desalting of oligonucleotides

Choosing the appropriate MWCO

Protein applications

For maximum retention, select a Nanosep device with a molecular weight cutoff that is three to six times smaller than the molecular weight of the molecule to be retained. For example, a 10K or 30K Nanosep device would be the appropriate selection for a 100K molecule. If rapid flow rate is required, choose a device with a MWCO at the lower end of this range (3×). If the main concern is retention, choose a tighter membrane (6×). See the table below for further guidance.

The molecular weight cutoff of an ultrafiltration (UF) membrane is defined by its ability to retain 90% of an ideally globular molecule. Many factors, including ionic conditions and protein:protein interaction, can affect the retention of biomolecules. We recommend that you pre-test retentivity of your biomolecular solution.

The table below describes MWCO selection for protein applications.

MWCO	Membrane nominal pore size[1]	Biomolecule size	Biomolecule molecular weight
3K	-	-	9K to 30K
10K	-	-	30K to 90K
30K	-	-	90K to 300K
100K	10 nm	30 to 90 nm	300K to 900K
300K	35 nm	90 to 200 nm	900K to 3000K

[1] Nominal pore size as measured by electron microscopy

The table below describes MWCO selection for virus applications.

MWCO	Membrane nominal pore size[1]	Virus or particle diameter
100K	10 nm	30 to 90 nm
300K	35 nm	90 to 200 nm

[1] Nominal pore size as measured by electron microscopy

DNA applications

During ultrafiltration, DNA in solution behaves differently than globular protein molecules. The table below provides guidelines for selecting the appropriate MWCO device for DNA concentration based on the size (in Kb) of the DNA molecule(s) being concentrated.

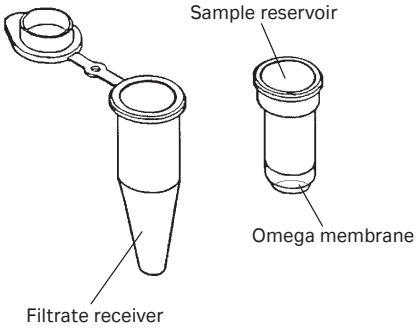
Note: *Speeds must be reduced to 5000 × g.*

The table below describes MWCO selection for nucleic acid applications.

MWCO	Base pairs (DS)	Bases (SS)
3K	16 to 50 Bp	32 to 95 Bs
10K	50 to 145 Bp	95 to 285 Bs
30K	145 to 475 Bp	285 to 950 Bs
100K	475 to 1450 Bp	950 to 2900 Bs
300K	1450 to 9500 Bp	2900 to 9500 Bs

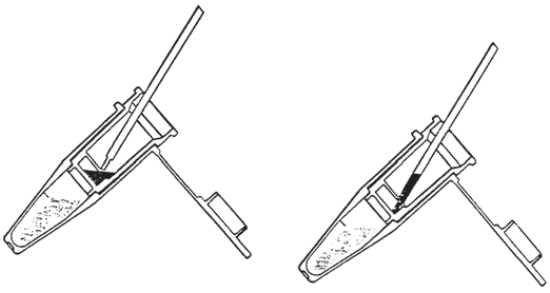
Components

Each Nanosep device consists of a sample reservoir with encapsulated membrane and a filtrate receiver.



Nanosep device operation

Note: *The Nanosep device has a very fast flow rate. Typically 4 to 20 minutes is sufficient to concentrate a sample. See Protein spin times, on page 3 to estimate the appropriate spin times.*

Step	Action
1	Make sure that the sample reservoir is firmly placed into the filtrate receiver.
2	Pipette 50 to 500 µL of sample into the sample reservoir. Cap the Nanosep device.
3	Place the Nanosep device into a fixed-angle centrifuge rotor that accepts 1.5 mL tubes. ***Note:*** *Always counterbalance the rotor with another Nanosep device containing an equivalent sample volume.*
4	Spin up to 14 000 × g for the required length of time (approximately 4 to 20 minutes) to achieve the desired concentrate volume. For maximal DNA retention, do not exceed 5000 × g.
5	At the end of spin time, stop the centrifuge and remove the Nanosep devices. Concentrated sample is recovered with a micropipette, as shown in the image below.  ***Note:*** *In some cases, it can appear as if the sample has "spun dry" in the device. This is not the case as some residual moisture always remains in the membrane. In this case, the sample is easily recovered by pipetting ~ 20 µL of buffer onto the membrane and recovering with a micropipette.*

Pre-rinsing (optional)

The Omega membrane in Nanosep devices contains trace amounts of glycerin (approximately 0.5 mg/device) and sodium azide (approximately 5.0 µg/device). If these chemicals interfere with an assay, they can be removed by filtering 500 µL of deionized water or buffer through the membrane two times. If further flushing is required, start with 0.05 N NaOH and repeat the procedure.

Note: *Do not allow the membrane to dry out prior to use. Dehydration can cause irreversible membrane damage.*

Nanosep concentration selection

The Nanosep concentration selection guide is meant to serve as a recommendation for concentrating protein samples. The total volume of liquid in the device determines the final retentate volume. By adding buffer in the filtrate receiver, you can set your dead stop volume and thereby select the concentration factor.

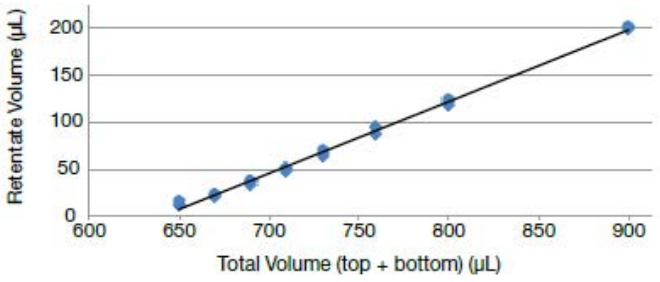
The following table shows what buffer volume must be added to the filtrate receiver under the insert to achieve desired concentration factors for 200, 300 and 400 µL starting sample volumes in the insert.

For instance, If you would like to concentrate 200 µL of starting material by ten fold (as shown in the table below), the buffer volume to be added to the filtrate receiver would be 470 µL, leaving 20 µL of concentrated material in the retentate.

Concentration factor (fold)	Starting sample volume (µL)	Volume added to filtrate receiver (µL)	Final retentate volume (µL)
2	200	572	100
3	200	530	67
4	200	508	50
5	200	496	40
6	200	487	33
10	200	470	20
20	200	470	10

Concentration factor (fold)	Starting sample volume (µL)	Volume added to filtrate receiver (µL)	Final retentate volume (µL)
25	200	455	8
2	300	536	150
3	300	472	100
4	300	440	75
5	300	421	60
6	300	408	50
10	300	383	30
20	300	364	15
25	300	360	12
2	400	500	200
3	400	415	133
4	400	372	100
5	400	347	80
6	400	330	67
10	400	296	40
20	400	270	20
25	400	265	16

The image below shows retentate volume dependence on total liquid volume in Nanosep centrifugal device with 30 K MWCO.



An IgG solution with 1 mg/mL concentration (0.1% w/v) prepared in 1× PBS buffer was used with 200, 300 and 400 µL sample volumes in insert and additional volume in filtrate receiver to achieve total volume indicated to and determine retentate volume in insert following centrifugation at 2500 × g for 20 minutes.

Note: *Results can vary based on your own experimental conditions.*

Protein spin times

Samples of 0.5 mL of a 1.0 mg/mL solution were centrifuged at 14 000 × g and were concentrated to a volume of 10 to 60 µL.

Solute	Solute MW	MWCO					
		3K	10K	30K	100K	300K	
		Spin time (min)					
		15	10	8	5	3	
Vitamin B12	1,335	7	-	-	-	-	% recovery
Aprotinin	6,200	99	51	11	-	-	% recovery
Cytochrome C	12,400	100	89	77	1.8	-	% recovery
Chymotrypsinogen A	25,000	-	97	94	2.1	-	% recovery
Ovalbumin	45,000	-	97	92	3	-	% recovery
BSA	67,000	-	-	100	26	1.5	% recovery
Phosphorylase B	97,400	-	-	95	91	1	% recovery
IgG	156,000	-	-	-	97	1.5	% recovery
Thyroglobulin (1 mg/mL)	677,000	-	-	-	100	91	% recovery

DNA spin times

Typically DNA solutions do not form gel layers and can be spun at 5000 × g for 5 to 10 minutes.

Sanitization

Nanosep devices can be sanitized by soaking or filtering 70% ethanol through the device.

Step	Action
1	Fill the sample reservoir with 70% ethanol.
2	Close the cap and centrifuge at 14 000 × g until all liquid passes through the membrane.
3	Discard the filtrate.
4	Remove residual ethanol by filling the device with sterile water and centrifuging again. ***Note:*** *Use the device within 20 minutes to prevent membrane dehydration.*

Chemical compatibility

• = Compatible x = Not compatible

Reagent	Device compatibility
Acetic Acid (10%)	•
Acetone (20%)	•
Acetonitrile (20%)	•
Ammonium Hydroxide (1 N)	•
Ammonium Sulfate	•
Chloroform (1%)	•
Dimethyl Sulfoxide (20%)	•
Dimethyl Formamide (20%)	•
Ethanol (70%)	•
Ethyl Acetate	x
Formaldehyde (5%)	•
Formic Acid (1 N)	•
Glycerol	•
Guanidine HCl (6 M)	•
Hydrochloric Acid (1 N)	•
Hydrogen Peroxide (10%)	•
Methanol (70%)	•
Methyl Ethyl Ketone (10%)	•
Phosphate Buffer	•
Phosphoric Acid (1 N)	•
Polyethylene Glycol (0.1%)	•
Propanol (70%)	•
Saline Buffer (0.85%)	•
Sodium Dodecyl Sulfate (0.01 M)	•

Reagent	Device compatibility
Sodium Hydroxide (1 N)	•
Sodium Hypochlorite (0.05%)	•
Sulfuric Acid (1 N)	•
Terg-a-zyme (1%)	•
Tris Buffer (1 M)	•
Ultrasil 11 (2%)	•
Urea (6 M)	•

Troubleshooting

Problem	Possible cause	Possible solution
Spin time too long	Centrifuge speed too slow	Increase g-force; re-calibrate centrifuge
	Gel layer buildup	Check rotors; fixed angle rotor recommended
	Biomolecule reached maximum concentration	Further concentration not possible
Loss of sample activity	Interference of trace amounts of glycerine and azide in Nanosep device	Follow pre-rinsing procedure, see *Pre-rinsing (optional), on page 2*
	Incompatibility of biomolecule and solvent	Adjust pH; change buffer type
	Protein:protein interactions, gel layer formation	Decrease final concentration factor (increase final concentrate volume)
Passage of biomolecule into filtrate	Poor retention of biomolecule by membrane	Select lower molecular weight cutoff
	Sub-units of biomolecule may be passing through membrane into filtrate	
Presence/interference of unanticipated molecules in sample	Retention of unanticipated molecule	Select higher molecular weight cutoff, allowing passage of interfering molecule
Recovery too low	Biomolecule not retained by membrane	Check for level of biomolecule in filtrate; if level is unacceptably high, choose a lower molecular weight cutoff
	g-force too high	Reduce g-force to 5,000 × g or less

Specifications

Parameter	Specification
Materials of construction	Filter media: Omega membrane (low protein-binding, modified polyethersulfone on polyethylene substrate) Filtrate receiver: Polypropylene
Effective filtration area	0.28 cm^2
Dimensions	Overall length (fully assembled): 4.5 cm (1.77 in.) with cap
Capacities	Maximum sample volume: 500 µL Final concentrate volume: 15 µL Filtrate receiver volume: 500 µL Hold-up volume (membrane/support): < 5 µL

Parameter	Specification
Operating temperature range	0°C to 40°C
pH range	2 to 14
Maximum centrifugal force	14 000 × g
Centrifuge	Fits rotors that accept 1.5 mL tubes
Sanitization	Provided non-sterile; may be sanitized by filtering 70% ethanol through the device prior to use

Ordering information

Description	24/pkg	100/pkg	500/pkg
3K, gray	OD003C33	OD003C34	OD003C35
10K, blue	OD010C33	OD010C34	OD010C35
30K, red	OD030C33	OD030C34	OD030C35
100K, clear	OD100C33	OD100C34	OD100C35
300K, orange	OD300C33	OD300C34	OD300C35

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

Nanosep MF (microfiltration) centrifugal devices are available with Bio-Inert™ or wwPTFE (hydrophilic PTFE) for low protein-binding and high recoveries in applications such as particulate removal prior to sample analysis (e.g. HPLC), removal of precipitates, and elution of sample from polyacrylamide gels.

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