Instructions for Use

Vivacon® 500 PCR Grade

Sterile, DNA Free*
For in vitro use only



DOMINIQUE DUTSCHER

85032-536-61

SARTORIUS

Vivacon® 500 µl PCR Grade – Introduction

Storage conditions | shelf life
Vivacon® ultrafiltration spin columns should be stored at room temperature. The devices should be used before the expiry date printed on the box.

Introduction
Vivacon® 500 PCR Grade concentrators are disposable ultrafiltration devices optimally suited for DNA and protein concentration. For optimal performance with DNA and protein samples, they are equipped with the patented regenerated cellulose membrane Hydrosart®.

Vivacon® 500 PCR Grade can be used in a benchtop fixed angle rotor, accepting 1.5 | 2.2 ml centrifuge tubes.

* Vivacon® 500 PCR Grade units are treated using a fully validated dual-cycle ethylene oxide (ETO) process. This renders the devices sterile and essentially free of human genomic DNA to an assurance level of < 2 pg; as tested by quantitative PCR amplification.

Equipment required for Vivacon® 500 PCR Grade

Centrifuge

Rotor type	Fixed angle
Minimum rotor angle	40°
Rotor cavity mm	To fit 1.5 | 2.2 ml/11 conical bottom tubes

Operation

1. When working with **DNA** samples, select a molecular weight cut off (MWCO) which retains the fragment size of double stranded DNA (ds DNA) as shown in Table 5. When working with **proteins**, select a MWCO at least 50% smaller than the molecular size of the protein of interest.

2. Fill concentrator with up to maximum volumes shown in Table 1. (Ensure lid is fully sealed).

3. Insert assembled concentrator into centrifuge.

4. Centrifuge at speeds recommended in Table 2, taking care not to exceed the maximum g force indicated by the MWCO.

5. Once the desired concentration is achieved, (see Table 3 or 4 for guide to concentration times), remove assembly and recover sample by reverse spinning the concentrate into a fresh collection tube. In this procedure remove filtrate tube, invert the concentrator body into new filtrate tube and then spin at up to 2,500 g for 2 minutes (or pulse for 20–30 seconds).
   The filtrate can be sealed for storage by closing the filtrate tube cap.

Note:
It is not possible to do a backflush with the device.

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Desalting | Buffer Exchange

1. Concentrate sample to desired level.
2. Empty filtrate container.
3. Refill concentrator with an appropriate solvent.
4. Concentrate the sample again and repeat the process until the concentration of contaminating microsolute is sufficiently reduced. Typically 3X wash cycles will remove 99% of initial salt concentration.

Vivacon® 500 PCR Grade Reverse Spinning

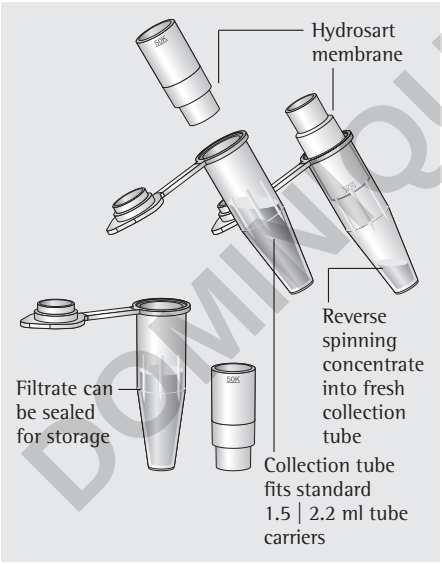


Table 1: Technical Specifications

Concentrator capacity	
Fixed angle rotor	0.5 ml
Dimensions	
Total length (Concentration)	45 mm
Total length (Back-spin)	47.5 mm
Width	12.4 mm
Active membrane area	0.32 cm^2
Hold-up volume	< 5 µl
Dead stop volume	5 µl (40° rotor)
Materials of construction	
Body	Polycarbonate
Filtrate vessel	Polypropylene
Membrane 2 kDa - 100 kDa 125 kDa	 Hydrosart® Cellulose Acetate (CA)
O-ring	Medical grade silicone

Table 2: Recommended Spin Speed in Fixed Angle Rotor (x g)

Membrane cut off	For DNA	For protein
2 kDa MWCO	7,500	14,000
10 kDa MWCO	7,500	14,000
30 kDa MWCO	5,000	14,000
50 kDa MWCO	5,000	14,000
100 kDa MWCO	3,000	8,000
125 kDa MWCO	2,500*	5,000

* Spin speed 2,500 × g for DNA samples > 900 bp when using a 125 kDa MWCO. For DNA samples >650 bp, spin at 1,000 × g.

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Usage Tips

1. Flow Rate
Filtration 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% glycerine will take up to 5 times longer to concentrate than samples in a predominantly buffer solution.

2. Pre-rinsing
Membranes fitted to Vivacon® concentrators contain trace amounts of glycerine. Should these interfere with analysis, they can be removed by rinsing fill volume of buffer solution or deionised water through the concentrator. Decant filtrate and concentrate before processing sample solution. If you do not want to use the pre-rinsed device immediately, store it in the refrigerator with buffer or water covering the membrane surface. Please do not allow the membrane to dry out.

3. Sterilisation of Vivacon® Devices
Vivacon® devices should not be autoclaved as high temperatures will substantially increase membrane MWCO.

4. Chemical Compatibility
Vivacon® concentrators are designed for use with biological fluids and aqueous solutions.

For chemical compatibility details, please refer to Table 6.

5. Retention and Recovery
The membranes used in Vivacon® are characterized by a molecular weight cut off (MWCO). For proteins, it corresponds to their ability to retain 90% of a molecule with this nominal molecular weight. For achieving better recovery, use a MWCO which is 1 to 2 of the species weight you need to concentrate.

For nucleic acid applications, strand length is the most useful parameter for selecting the Vivacon® device appropriate for a specific application. However, other parameters including DNA concentration, the magnitude of the driving force (g-force) and the salt concentration all act in concert to affect DNA recovery. For characteristic recoveries and concentration times, see Table 3 and 4. For the correlation between MWCO and nucleotide cut-off (bp), see Table 5.

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Performance Characteristics

Table 3: Performance Characteristics Vivacon® 500 PCR Grade for DNA
Start volume 0.5 ml, sample concentration 50 ng/ml

	Sample size (bp)	Time to concentrate up to 30x [min.] at 20°C	Concentrate recovery %	g-force (xg)
2,000 MWCO	10	60 min	93%	7,500
10,000 MWCO	30	25 min	94%	7,500
30,000 MWCO	50	18 min	88%	5,000
50,000 MWCO	300	18 min	91%	5,000
100,000 MWCO	600	10 min	87%	3,000
125,000 MWCO	650	12 min	85%	2,000
125,000 MWCO	900	9 min	94%	3,000

Table 4: Performance Characteristics Vivacon® 500 PCR Grade for proteins
Start volume 0.5 ml, sample and concentration of proteins as specified in table

	Sample	Time to concentrate up to 30x [min.] at 20°C	Concentrate recovery %	g-force (xg)
2,000 MWCO	0.25 mg/ml cytochrome c	30 min	95%	14,000
10,000 MWCO	0.25 mg/ml cytochrome c	15 min	92%	14,000
30,000 MWCO	1.0 mg/ml BSA	10 min	95%	14,000
50,000 MWCO	1.0 mg/ml BSA	10 min	92%	14,000
100,000 MWCO	1.0 mg/ml bovine IgG	11 min	90%	8,000
125,000 MWCO	1.0 mg/ml bovine IgG	10 min	81%	8,000

Table 5: Conversion Table for Hydrosart® MWCO to Nucleotide Cut-off

Membrane	MWCO	Double-Stranded Nucleotide Cut-off (bp)
Hydrosart®	2 kDa	> 10
Hydrosart®	10 kDa	> 30
Hydrosart®	30 kDa	> 50
Hydrosart®	50 kDa	> 300
Hydrosart®	100 kDa	> 600
Cellulose Acetate	125 kDa	> 650

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Table 6: Chemical Compatibility (2hr contact time)

	Hydrosart®	Cellulose Acetate
Compatible pH range	pH 1-9	pH 4-8
Acetic Acid (25.0%)	OK	NO
Acetone (10.0%)	NO	NO
Acetonitrile (10.0%)	NO	NO
Ammonium Hydroxide (5.0%)	OK	OK
Benzene (100%)	NO	NO
Chloroform (1%)	OK	OK
Dimethyl Formamide (10.0%)	NO	NO
Dimethyl Sulfoxide (5.0%)	NO	NO
EDTA (1.0 M)	Yes	Yes
Ethanol (70.0%)	OK	OK
Ethyl Acetate (100%)	NO	NO
Formaldehyde (30%)	OK	OK
Formic Acid (5.0%)	OK	?
Glycerine (70%)	OK	OK
Guanidine HCl (6 M)	OK	?
Hydrocarbons, aromatic	NO	NO
Hydrocarbons, chlorinated	NO	NO
Hydrochloric Acid (1 M)	OK	NO
Isopropanol (70%)	OK	OK
Lactic Acid (5.0%)	OK	NO
Mercaptoethanol (1.0 M)	OK	NO
Methanol (60%)	OK	OK
Nitric Acid (10.0%)	NO	NO
Phenol (1%)	OK	OK
Phosphate Buffer (1.0 M)	OK	OK
Sodium Chloride (5M)	Yes	Yes
Sodium Dodecylsulfate (0.1 M)	OK	OK
Sodium Hydroxide (1.0 M)	NO	NO
Sodium Hypochlorite (200 ppm)	NO	NO
Sodium Nitrate (1.0%)	OK	?
Tetrahydrofuran (5.0%)	NO	NO
Toluene (1.0%)	NO	NO
Trifluoroacetic Acid (10%)	OK	NO
TRIS Buffer pH 7,2 - 9 (1.0 M)	Yes	Yes
Tween 20 (0.1%)	OK	OK
Triton X-100 (0.1%)	OK	OK
Urea (8 M)	OK	?

OK = Acceptable ? = Questionable NO = Not recommended

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FAQ

– DNA recovery is lower than expected.
If the DNA sample contains a high salt concentration, dilute the sample.
Run the device at the recommended g-force.

– Can proteins be concentrated with Vivacon®?
Proteins can be concentrated with Vivacon®, using the guidelines on page 5 to choose the correct MWCO. However, we recommend Vivaspin® 500 for protein concentration due to faster concentration achieved with a vertical membrane design for protein applications.

– Sample runs to dryness.
Spinning for much longer than the recommended spin times can allow samples to go to dryness. To recover the sample, add 10 µl of water or buffer to the device, vortex gently for up to1 min and then recovery as normal.

– DNA recovery too low.
Spinning the sample at 1000 × g may result in higher DNA recoveries, when working close to the membrane cut off limits, e.g. with a 650 bp DNA sample with a 125 kDa membrane.

– No DNA signal visible after PCR reaction.
Using membrane cut offs smaller than 125 kDa can in some cases lead to concentration of PCR inhibitors along with the sample DNA. Use the 125 kDa membrane cut off and spin your samples at 2000 x g for optimal DNA recovery and sequencing results.
Additionally, 1-2 washes with buffer may be needed to remove the inhibitors.

Ordering Information

Vivacon® 500 PCR Grade	Qty. per box	Prod. No.
30,000 MWCO	25	VN01H21ETO
30,000 MWCO	100	VN01H22ETO
30,000 MWCO	500	VN01H23ETO
50,000 MWCO	25	VN01H31ETO
50,000 MWCO	100	VN01H32ETO
50,000 MWCO	500	VN01H33ETO
100,000 MWCO	25	VN01H41ETO
100,000 MWCO	100	VN01H42ETO
100,000 MWCO	500	VN01H43ETO
125,000 MWCO	25	VN01H81ETO
125,000 MWCO	100	VN01H82ETO
125,000 MWCO	500	VN01H83ETO

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