

Instructions for Use

Vivacon[®] 2 PCR Grade

Sterile, DNA Free*

For in vitro use only



85032-536-60



SARTORIUS

Vivacon® 2 ml 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® 2 PCR Grade concentrators are disposable ultrafiltration devices optimally suited for DNA concentration. For optimal performance with DNA samples, they are equipped with the patented regenerated cellulose membrane Hydrosart®.

Vivacon® 2 PCR Grade is suitable for sample volumes of 100 µl to 2 ml. Vivacon® 2 PCR Grade can effectively be used in a fixed angle rotor accepting 15 ml centrifuge tubes.

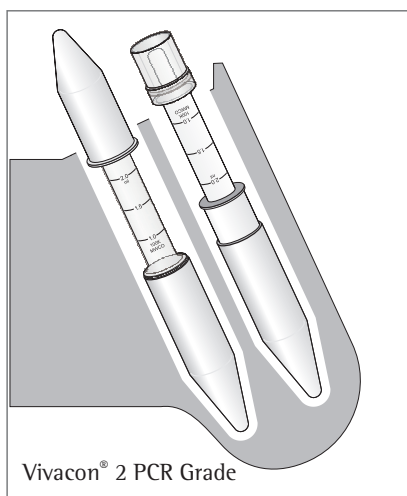
Vivacon® 2 PCR Grade is specifically designed with low internal surface and membrane area and allows a re-spin collection of the concentrate, in order to achieve superior recoveries from very dilute samples.

* Vivacon® 2 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 Vivacon® 2 PCR Grade

| Centrifuge | Vivacon® 2 PCR Grade |
|---------------------|--|
| Rotor type | Fixed angle |
| Minimum rotor angle | 25° |
| Rotor cavity | To fit 17 × 120 mm 15 ml conical bottom tubes (recommended cavity depth 95 mm) |

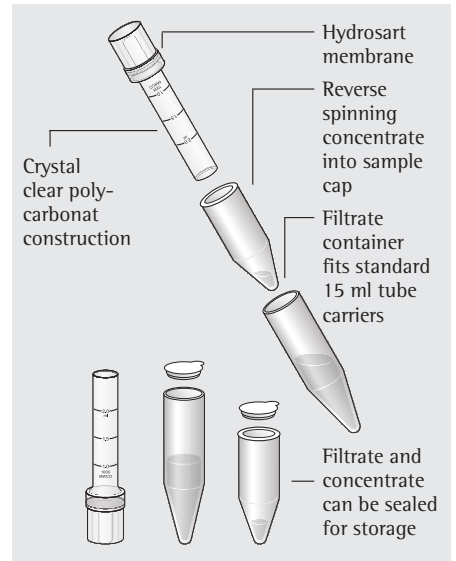
The backspin cap ridge must be below the top of the rotor cavity for correct support during centrifugation.



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 4. 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 for guide to concentration times), remove assembly and recover sample by reverse spinning the concentrate into the recovery cap. In this procedure remove filtrate tube, invert the concentrator body, insert concentrate recovery cap into filtrate tube and then spin at up to 2,500 g for 2 minutes. The concentrate recovery cap can be sealed for storage.

Vivacon® 2 PCR Grade Reverse Spinning



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 microsoluble is sufficiently reduced. Typically 3X wash cycles will remove 99% of initial salt concentration.

Technical Specifications

Table 1: Technical Specifications

| Concentrator capacity | Vivacon® 2 PCR Grade |
|----------------------------------|-----------------------------|
| Fixed angle rotor | 2.0 ml |
| Dimensions | |
| Total length (Concentration) | 125 mm |
| Total length (Back-spin) | 115 mm |
| Width | 16 mm |
| Active membrane area | 0.95 cm ² |
| Hold-up volume | 10 µl |
| Dead stop volume | 55 µl (25° rotor) |
| Materials of construction | |
| Body | Polycarbonate |
| Filtrate vessel | Polypropylene |
| Back spin vial | Polypropylene |
| Concentrator cap | Polypropylene |
| Membrane | |
| 2 kDa - 100 kDa | Hydrosart® |
| 125 kDa | Cellulose acetate (CA) |
| O-ring | Silicone |

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

| Device | Vivacon® 2 | |
|-------------------------|-------------------|---------------------|
| Membrane cut off | For DNA | For proteins |
| 30 kDa MWCO | 2,500 | 5,000 |
| 50 kDa MWCO | 2,500 | 5,000 |
| 100 kDa MWCO | 2,500 | 5,000 |
| 125 kDa MWCO | 2,500* | 5,000 |

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

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 5.

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 3a and 3b. For the correlation between MWCO and nucleotide cut-off (bp), see Table 4.

Performance Characteristics

Table 3a: Performance Characteristics Vivacon® 2 PCR Grade for DNA

Start volume 2 ml, sample concentration 50 ng/ml

| | Sample size (bp) | Time to concentrate up to 30x [min.] at 20°C | Concentrate recovery % | g-force (xg) |
|--------------|------------------|--|------------------------|--------------|
| 30,000 MWCO | 50 | 60 min | 95% | 2,500 |
| 50,000 MWCO | 300 | 45 min | 96% | 2,500 |
| 100,000 MWCO | 600 | 30 min | 93% | 2,500 |
| 125,000 MWCO | 650 | 30 min | 88% | 2,500 |
| 125,000 MWCO | 900 | 30 min | 89% | 2,500 |

Table 3b: Performance Characteristics Vivacon® 2 PCR Grade for Proteins

Start volume 2 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) |
|--------------|----------------------|--|------------------------|--------------|
| 30,000 MWCO | 1.0 mg/ml BSA | 40 min | 96% | 5,000 |
| 50,000 MWCO | 1.0 mg/ml BSA | 30 min | 94% | 5,000 |
| 100,000 MWCO | 1.0 mg/ml bovine IgG | 30 min | 92% | 5,000 |
| 125,000 MWCO | 1.0 mg/ml bovine IgG | 27 min | 81% | 5,000 |

Table 4: Conversion Table for Hydrosart® MWCO to Nucleotide Cut-off

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

Table 5: 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 |
| 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 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 |
| Tween 20 (0.1%) | OK | OK |
| Triton X-100 (0.1%) | OK | OK |
| Urea (8 M) | OK | ? |

OK = Acceptable

? = Questionable

NO = Not recommended

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® 2 for protein concentration due to faster concentration achieved with a vertical membrane design for protein applications.

– DNA recovery too low.

Spinning the sample at $1000 \times g$ 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 $2000 \times g$ for optimal DNA recovery and sequencing results.

Additionally, 1-2 washes with buffer may be needed to remove the inhibitors.

Ordering Information

| Vivacon® 2 PCR Grade | Qty. per box | Prod. No. |
|-----------------------------|---------------------|------------------|
| 30,000 MWCO | 25 | VN02H21ETO |
| 30,000 MWCO | 100 | VN02H22ETO |
| 30,000 MWCO | 500 | VN02H23ETO |
| 50,000 MWCO | 25 | VN02H31ETO |
| 50,000 MWCO | 100 | VN02H32ETO |
| 50,000 MWCO | 500 | VN02H33ETO |
| 100,000 MWCO | 25 | VN02H41ETO |
| 100,000 MWCO | 100 | VN02H42ETO |
| 100,000 MWCO | 500 | VN02H43ETO |
| 125,000 MWCO | 25 | VN02H81ETO |
| 125,000 MWCO | 100 | VN02H82ETO |
| 125,000 MWCO | 500 | VN02H83ETO |

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