



sartorius stedim  
biotech

## Ultrafiltration & Protein Purification Products



**Fisher Scientific**



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## Major uses for ultrafiltration

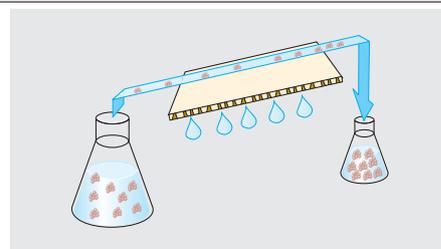
Ultrafiltration is a convective process that uses anisotropic semi-permeable membranes to separate macromolecular species and solvents primarily on the basis of size. It is particularly appropriate for the concentration of macromolecules and can also be used to purify molecular species or for solvent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

### Typical applications for ultrafiltration

- Concentration | desalting of proteins, enzymes, DNA, monoclonal antibodies, immunoglobulins
- Free drug, hormone assays
- Removal of primers from PCR amplified DNA
- Removal of labelled amino acids and nucleotides
- HPLC sample preparation
- Deproteinization of samples
- Recovery of biomolecules from cell culture supernatants, lysates
- General purpose laboratory concentration and desalting of proteins, enzymes, cells, DNA, biomolecules, antibodies and immunoglobulins
- Mammalian cell harvesting
- Cell washing, virus purification, cell debris removal, depyrogenation

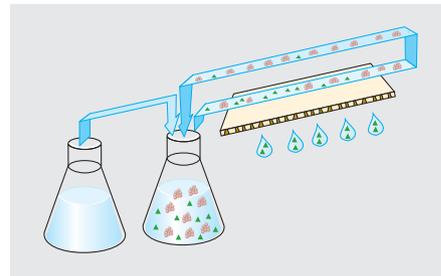
#### Solute concentration

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.



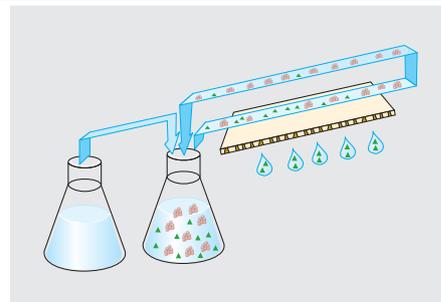
#### Solute fractionation or clarification

Ultrafiltration is a cost effective method for separating samples into size-graded components providing that macromolecular fractions differ in size by a 10x MW difference. During filtration, the permeating solute remains at its initial concentration whilst the retained macromolecules will be enriched.



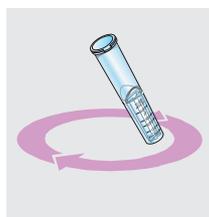
#### Solute desalting or purification

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. Microsolute is removed most efficiently by adding solvent to the solution being ultrafiltered at a rate equal to the speed of filtration. This is called diafiltration.

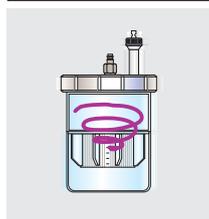


## Process alternatives

Sartorius Stedim Biotech offers a comprehensive range of process alternatives for the filtration and concentration of biological samples. Below is a guide to selecting the most suitable filtration method, depending on sample volume, equipment available, filtration speed and process control desired.



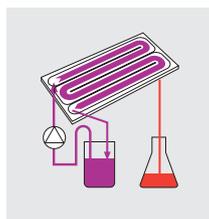
**Centrifugal concentrators** (100  $\mu$ l to 100 ml starting volumes)  
Centrifugation provides the vector to clear solvent and micro molecules through the ultrafiltration membrane and into a filtrate container positioned below. This is a gentle process that is characterised by quick set up and fast filtration speeds with most solutions. Sartorius Stedim Biotech offers ten alternative centrifugal devices covering volumes from 100  $\mu$ l up to 100 ml.



**Pressure ultrafiltration** (5 to 250 ml starting volume)  
Pressurised air or an inert gas is used to provide the filtration vector. For fastest filtration, Vivacell products are used with an orbital laboratory shaker. Agitation is used to impede macromolecules from polarising on the membrane surface and reducing filtration speed. Vivaspin 20, Vivacell 70, Vivacell 100 and Vivacell 250 can be run with gas pressure.



Pressure-fugation is a unique Sartorius Stedim Biotech method that combines gas pressure with centrifugation. This is the fastest concentration method with process times typically 30 to 50% faster than centrifugation alone. Vivaspin 20 and Vivacell 70 can be operated this way (5 to 50 ml starting volumes).



**Cross flow** (100 ml to several litres starting volume)  
The solution to be processed is pumped under pressure across an ultrafiltration membrane and then returned to the original reservoir. The solution is progressively concentrated or purified as solvent and micro-molecules pass through the membrane into a separate filtrate vessel. Vivaflow 50 and 200 are offered for this procedure.



**Solvent absorption** (1 to 20 ml starting volume)  
This technique uses an absorbent cellulose pad mounted behind the ultrafiltration membrane to draw solvents and micro solutes through the membrane. Retained macromolecules are concentrated into the bottom of the sample container. No additional equipment is required. Five Vivapore devices are offered for this procedure with maximum initial sample volumes ranging from 1 to 20 ml.

## Membrane performance characteristics

Sartorius Stedim Biotech offers an extended range of membranes to cover the great majority of ultrafiltration requirements.

The following is a guide to selecting the most appropriate membranes according to their typical performance characteristics. Please note however, that membrane behaviour and ultimate performance, largely depends on the specific characteristics of the solution being processed. Sartorius Stedim Biotech recommends that users experiment with alternative membranes in order to optimise their process performance.

### Polyethersulfone (PES)

This is a general purpose membrane that provides excellent performance with most solutions when retentate recovery is of primary importance. Polyethersulfone membranes are usually preferred for their low fouling characteristics, exceptional flux and broad pH range.

### Cellulose triacetate (CTA)

High hydrophilicity and very low non specific binding characterise this membrane. Cast without any membrane support that could trap or bind passing micro solutes, these membranes are preferred for sample cleaning and protein removal and when high recovery of the filtrate solution is of primary importance.

### Hydrosart®

Hydrosart demonstrates the same properties as regenerated cellulose, but with the added benefit of enhanced performance characteristics and extremely low protein binding, making it the membrane of choice for applications such as concentration and desalting of immunoglobulin fractions.

## Membrane performance comparisons

Membrane	Frequently preferred for:
Polyethersulfone 3,000 MWCO 5,000 MWCO 10,000 MWCO 30,000 MWCO 50,000 MWCO 100,000 MWCO	Concentration   desalting of column eluates
Cellulose triacetate 5,000 MWCO 10,000 MWCO 20,000 MWCO	Free   bound drug studies; whenever the filtrate is being analysed
Hydrosart® 2,000 MWCO 5,000 MWCO 10,000 MWCO 30,000 MWCO	Concentration   desalting of column eluates. Hydrosart Membrane evaluation for upscaling.

## Membrane selection guide

The advanced designs and low adsorption materials that characterise Sartorius Stedim Biotech products, offer a unique combination of faster processing speeds and higher recovery of the concentrated sample. Providing that the appropriate device size and membrane cut-off is selected, Sartorius Stedim Biotech products will typically yield recoveries of the concentrated sample in excess of 90% when the starting sample contains over 0.1 mg/ml of the solute of interest. Most of the loss is caused by non specific binding both to the membrane surface and to exposed binding sites on the plastic of the sample container:

### Adsorption to the membrane

Depending on sample characteristics relative to the membrane type used, solute adsorption on the membrane surface is typically 2-10 µg/cm<sup>2</sup>. This can increase to 20-100 µg/cm<sup>2</sup> 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 alternative.

### Adsorption to the sample container

Although every effort is made to minimise this phenomenon by the selection of low adsorption materials and tool production to optical standards, some solute will bind to the internal surface of the sample container.

Whilst the relative adsorption will be proportionately less important than on the membrane, due to the higher total surface area, this can be the major source of yield loss.

### Process optimisation

When highest recoveries are most important, in particular when working with solute quantities in the microgram range, Sartorius Stedim Biotech recommends that users consider the following:

- Select the smallest device that suits the sample volume. Additionally, take advantage of the extra speed of Sartorius Stedim Biotech products by refilling a smaller device repeatedly.
- Select the lowest MWCO membrane that suits the application.
- Reduce pressure or centrifugal force to approximately half of the maximum recommended.
- 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.
- Pretreat the device overnight with a passivation solution such as 5% SDS, Tween 20, or Triton X in distilled water. Then rinse thoroughly before use.

### Membrane selection guide (recommended MWCO)

Application	< 5,000	10,000	30,000	50,000	100,000	> 300,000
Bacteria					•	•
DNA fragments		•	•	•	•	
Enzymes	•	•				
Growth factors	•	•				
Immunoglobulins			•	•	•	
Nucleic Acids	•	•	•	•	•	
MAB			•	•	•	
Oligonucleotides	•					
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

## Vivaspin 500



### 100 $\mu$ l to 500 $\mu$ l samples

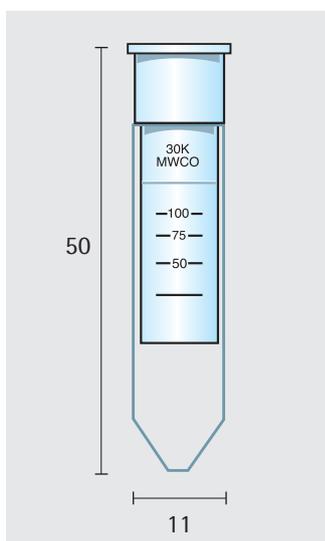
Vivaspin 500  $\mu$ l centrifugal filter units offer a simple, one step procedure for sample preparation. They can effectively be used in a fixed angle rotors accepting 2.2 ml centrifuge tubes.

The patented vertical membrane design and thin channel filtration chamber (US 5,647,990), minimises membrane fouling and provides high speed concentrations, even with particle laden solutions.



### Technical specifications Vivaspin 500

Concentrator capacity	Swing bucket rotor	do not use
	Fixed angle rotor	500 $\mu$ l
Dimensions	Total length	50 mm
	Width	11 mm
	Active membrane area	0.5 cm <sup>2</sup>
	Hold-up volume, membrane and support	< 5 $\mu$ l
	Dead stop volume	5 $\mu$ l
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polypropylene
	Concentrator cap	Polycarbonate
	Membrane	Polyethersulfone



### Equipment required Vivaspin 500

Centrifuge	Rotor type	Fixed angle
	Minimum rotor angle	40°
	Rotor cavity	To fit 2.2 ml (11 mm) conical bottom tubes
	Maximum speed	15,000 g
Concentrate recovery	Pipette type	Fixed or variable volume
	Recommended tip	Thin gel loader type

**Performance characteristics**

	Time to concentrate up to 30x [min.] at 20°C and solute recovery %	
	Min.	Rec.
Rotor	Fixed angle	
Centrifugal force	12,000 g	
Start volume	500 µl	
Aprotinin 0.25 mg/ml (6,500 MW) 3,000 MWCO PES	30	96%
BSA 1.0 mg/ml (66,000 MW) 5,000 MWCO PES	15	96%
10,000 MWCO PES	5	96%
30,000 MWCO PES	5	95%
IgG 0.25 mg/ml (160,000 MW) 30,000 MWCO PES	10	96%
50,000 MWCO PES	10	96%
100,000 MWCO PES	10	96%

**Ordering information**

Vivaspin 500 Polyethersulfone	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
3,000 MWCO	25	VS0191	14-558-412
3,000 MWCO	100	VS0192	14-558-413
5,000 MWCO	25	VS0111	14-558-398
5,000 MWCO	100	VS0112	14-558-399
10,000 MWCO	25	VS0101	14-558-396
10,000 MWCO	100	VS0102	14-558-397
30,000 MWCO	25	VS0121	14-558-400
30,000 MWCO	100	VS0122	14-558-401
50,000 MWCO	25	VS0131	14-558-402
50,000 MWCO	100	VS0132	14-558-403
100,000 MWCO	25	VS0141	14-558-404
100,000 MWCO	100	VS0142	14-558-405
300,000 MWCO	25	VS0151	14-558-406
300,000 MWCO	100	VS0152	14-558-407
1,000,000 MWCO	25	VS0161	14-558-408
1,000,000 MWCO	100	VS0162	14-558-409
0.2 µm	25	VS0171	14-558-410
0.2 µm	100	VS0172	14-558-411
Starter pack (5 of each 5 k, 10 k, 30 k, 50 k, 100 k)	25	VS01S1	14-558-416

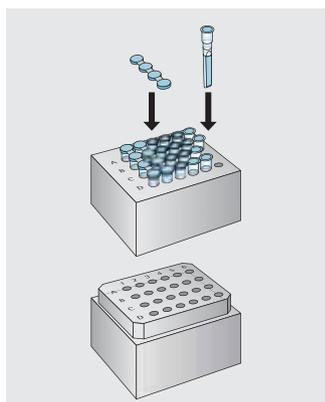
## 24-Well Ultrafiltration Frame

### Safe and fast protein concentration in high throughput format



The unique and reusable 24-well is designed to be fitted with up to 24 individual Vivaspin 500 ultrafiltration devices. The vertical membrane design and built in dead stop pocket inherent to all Vivaspin devices allow fast and safe high throughput concentration of 24 samples per plate.

The 24-well ultrafiltration frame and the supplied collection plates can effectively be used in a swing-out rotor for stacked deep well plates.



#### Vivaspin 500-HT

Vivaspin 500-HT centrifugal concentrators are designed for use with the Vivaspin 24-well ultrafiltration frame. The cap strips allow simple and convenient processing of 2–48 samples in parallel using a multiwell plate rotor accepting 2 stacked deep multiwell plates per bucket, and capable of spinning at up to 1,500 g.

Traditional Vivaspin 500 devices can be used in the 24-well ultrafiltration frame as well for a larger MWCO option.

#### Technical specifications 24-well ultrafiltration frame

Centrifuge requirements	Rotor type	Swing-out multiwell plate rotor accepting stacked deep well plates
	Maximum speed	1,500 g
Dimensions	Frame dimension (L × W × H)	128 × 85 × 25 mm
	Max. height of frame plus filtrate collection plate	49 mm
Materials of construction	Frame	Acetal
	Filtrate collection plate	Polystyrene
Concentrate recovery	Pipette type	Fixed or variable volume
	Recommended tip	Thin gel loader type

#### Performance characteristics

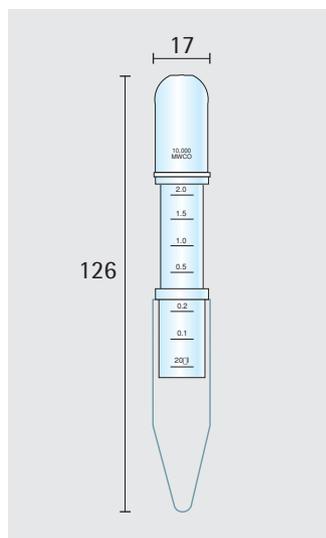
	Time to concentrate up to 30x [min.] and solute recovery %	
Rotor	Swing-out	
Centrifugal force	1,500 g	
Start volume	500 µl	
	Min.	Rec.
BSA 1.0 mg/ml (66,000 MW)		
10,000 MWCO PES	15	95%
30,000 MWCO PES	15	93%

**Ordering information**

	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
24-well ultrafiltration frame (includes 2 collection plate)	2	VW24HT051	14-558-586
24-well filtrate collection plate	12	VW24PS0212	14-558-587
Vivaspin 500 High Throughput (HT) Polyethersulfone (includes 120 cap strips)			
10,000 MWCO	480	VS01HT01	14-558-414
30,000 MWCO	480	VS01HT21	14-558-415

## Vivaspin 2

### Choice of membranes



#### 0.4–2 ml samples

The Vivaspin 2 bridges the gap between the 500  $\mu$ l and 4 ml centrifugal concentrators. This device combines the speed of the classic Vivaspin products with low internal surface and membrane area for superior recoveries from very dilute solutions.

Available with a choice of PES, Cellulose Triacetate and Hydrosart® membranes, Vivaspin 2 offers the highest flexibility for process optimisation.

Also unique to the Vivaspin 2, is the choice of directly pipetting the concentrate from the dead stop pocket built into the bottom of the concentrator, or alternatively reverse spinning into the concentrate recovery cap which can then be sealed for storage. Both methods result in near total concentrate recoveries.

### Technical specifications Vivaspin 2

Concentrator capacity	Swing bucket rotor	3 ml
	Fixed angle rotor	2 ml
Dimensions	Total length	126 mm
	Width	17 mm
	Active membrane area	1.2 cm <sup>2</sup>
	Hold-up volume of membrane	<10 $\mu$ l
	Dead stop volume	8 $\mu$ l
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polycarbonate
	Concentrator cap	Polycarbonate
	Membrane	PES, CTA, HY

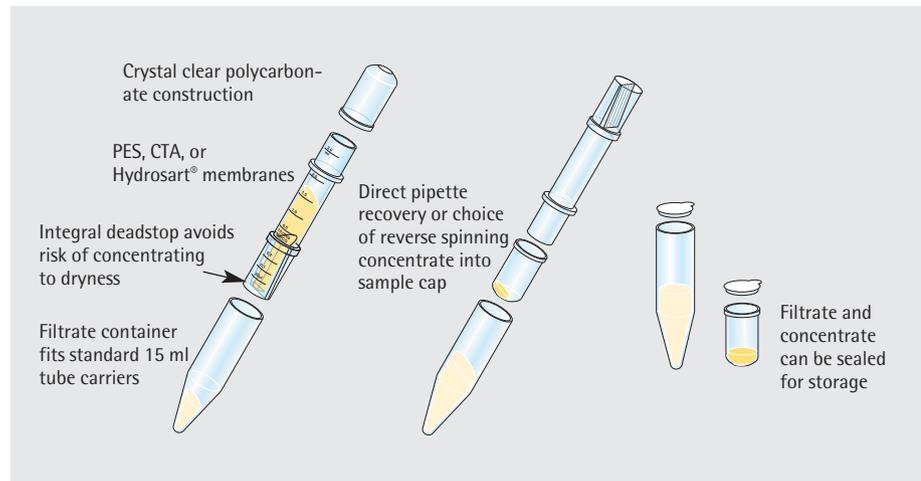
### Equipment required Vivaspin 2

Centrifuge		
Rotor type	Swing bucket	Fixed angle
Minimum rotor angle	–	25°
Rotor cavity	To fit 15 ml (17 mm) conical bottom tubes	To fit 15 ml (17 mm) conical bottom tubes
Maximum speed	4,000 g	12,000 g*
Concentrate recovery		
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type

\* Please note, devices with membrane MWCO >100 kDa need to be processed at lower g forces. See data sheets for details.

**Performance characteristics**

	Time to concentrate up to 30x [min.] at 20°C and solute recovery %	
	Min.	Rec.
Rotor	Fixed angle	
Centrifugal force	5,000 g	
Start volume	2 ml	
<b>Aprotinin 0.25 mg/ml (6,500 MW)</b>		
3,000 MWCO PES	50	96%
<b>BSA 1.0 mg/ml (66,000 MW)</b>		
5,000 MWCO PES	12	98%
5,000 MWCO CTA	50	96%
5,000 MWCO Hydrosart	22	98%
10,000 MWCO PES	8	98%
10,000 MWCO CTA	10	96%
10,000 MWCO Hydrosart	12	98%
20,000 MWCO CTA	5	96%
30,000 MWCO PES	8	97%
30,000 MWCO Hydrosart	5	97%
<b>IgG 0.25 mg/ml (160,000 MW)</b>		
20,000 MWCO CTA	6	97%
30,000 MWCO PES	10	96%
50,000 MWCO PES	10	96%
100,000 MWCO PES	8	95%



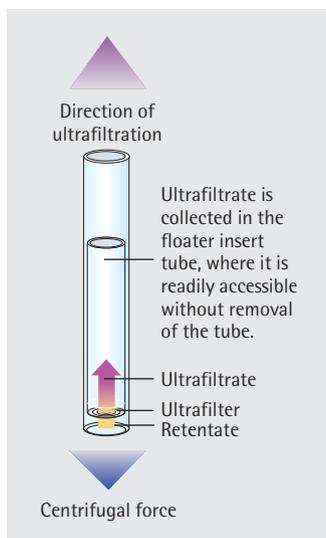
**Ordering information**

<b>Vivaspin 2 Polyethersulfone</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
3,000 MWCO	25	VS0291	14-558-433
3,000 MWCO	100	VS0292	14-558-434
5,000 MWCO	25	VS0211	14-558-419
5,000 MWCO	100	VS0212	14-558-420
10,000 MWCO	25	VS0201	14-558-417
10,000 MWCO	100	VS0202	14-558-418
30,000 MWCO	25	VS0221	14-558-421
30,000 MWCO	100	VS0222	14-558-422
50,000 MWCO	25	VS0231	14-558-423
50,000 MWCO	100	VS0232	14-558-424
100,000 MWCO	25	VS0241	14-558-425
100,000 MWCO	100	VS0242	14-558-426
300,000 MWCO	25	VS0251	14-558-427
300,000 MWCO	100	VS0252	14-558-428
1,000,000 MWCO	25	VS0261	14-558-429
1,000,000 MWCO	100	VS0262	14-558-430
0.2 µm	25	VS0271	14-558-431
0.2 µm	100	VS0272	14-558-432
Starter pack (5 of each 5 k, 10 k, 30 k, 50 k, 100 k)	25	VS02S1	14-558-443
<b>Vivaspin 2 Cellulose triacetate</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
5,000 MWCO	25	VS02U1	14-558-444
5,000 MWCO	100	VS02U2	14-558-445
10,000 MWCO	25	VS02V1	14-558-446
10,000 MWCO	100	VS02V2	14-558-447
20,000 MWCO	25	VS02X1	14-558-448
20,000 MWCO	100	VS02X2	14-558-449
<b>Vivaspin 2 Hydrosart</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
2,000 MWCO	25	VS02H91	14-558-441
2,000 MWCO	100	VS02H92	14-558-442
5,000 MWCO	25	VS02H11	14-558-437
5,000 MWCO	100	VS02H12	14-558-438
10,000 MWCO	25	VS02H01	14-558-435
10,000 MWCO	100	VS02H02	14-558-436
30,000 MWCO	25	VS02H21	14-558-439
30,000 MWCO	100	VS02H22	14-558-440

**Ordering Tips**

- Choose a membrane pore size at least 50% smaller than the size of the molecule to be retained.
- Usually choose Polyethersulfone membranes for fastest concentrations.
- Usually choose Cellulose Triacetate for Protein Removal | Ultrafiltrate recovery.
- Usually choose Hydrosart® membranes for highest recovery with Ig fractions.

## Centrisart I



### 0.5–2.5 ml samples

Centrisart I is a ready to use unit for small volume centrifugal ultrafiltration to separate proteins from low molecular weight substances in biological samples.

Centrisart I features a unique design, ultrafiltration in the opposite direction to the centrifugal force. This is so effective in preventing premature blockage of the filter that even whole blood samples can be deproteinized. The ultrafiltrate is collected in the floater insert tube, where it is readily accessible without removing the tube.

### Typical applications include:

- drug binding studies
- determination of metabolites in serum
- protein removal from blood samples
- cleaning of liposomes
- virus removal

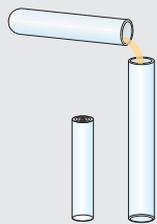
### Technical specifications Centrisart I

Concentrator capacity	Swing bucket rotor	2.5 ml
	Fixed angle rotor	2.5 ml
Dimensions	Total length	93 mm
	Width	14 mm
	Active membrane area	0.79 cm <sup>2</sup>
	Hold-up volume of membrane	< 5 µl
	Dead stop volume	100 µl
Materials of construction	Centrifuge tube	Polystyrene
	Floater tube	Cellulose propionate
	Cap	Polyethylene
	Membrane	CTA, PES

### Equipment required Centrisart I

<b>Centrifuge</b>		
Rotor type	Swing bucket	Fixed angle
Minimum rotor angle	–	25°
Rotor cavity	To fit 15 ml (17 mm) conical bottom tubes	To fit 15 ml (17 mm) conical bottom tubes
Maximum speed	2,500 g	2,000 g
<b>Concentrate recovery</b>		
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type

Easy-to-use



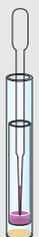
Remove interior tube, pour in sample



Replace interior tube



Centrifuge



Pipette out the filtrate...



...or use forceps to remove the interior tube to access the concentrate

Performance characteristics

	Time to filter 50% of sample volume	Time to filter 90% of sample volume	Passage of sample species volume
BSA 1.0 mg/ml (66,000 MW)			
5,000 MWCO	300 min	N   A	0%
10,000 MWCO	35 min	80 min	2%
20,000 MWCO	9 min	20 min	2%
IgG 0.25 mg/ml (160,000 MW)			
100,000 MWCO	13 min	35 min	3%
Blue Dextran 0.1 mg/ml (2,000,000 MW)			
300,000 MWCO	9 min	25 min	28%

\* 2.5 ml samples were loaded into each device.

The devices were centrifuged at 2,000 g until the required filtrate volumes had been reached.

## Ordering information

	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
5,000 MWCO CTA	12	13229-----E	14-558-220
10,000 MWCO CTA	12	13239-----E	14-558-221
20,000 MWCO CTA	12	13249-----E	14-558-222
100,000 MWCO PES	12	13269-----E	14-558-223
300,000 MWCO PES	12	13279-----E	14-558-224
Starter pack (3 units each of 5k, 10k, 20k, 100k)	12	13209-----E	14-558-219

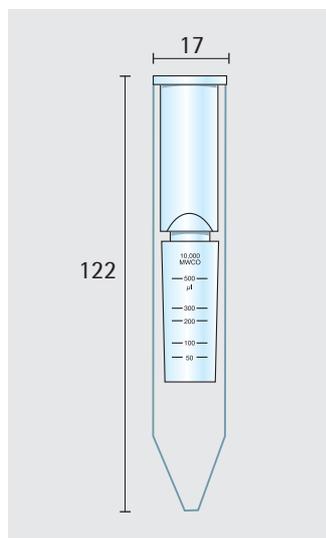
## References

P. Nebinger and M. Koel  
Determination of acyclovir by  
ultrafiltration and high-performance  
liquid chromatography.  
J. Chromatography 619, 342-344 (1993)

F. da Fonseca-Wollheim, K.-G. Heinze,  
K. Lomsky and H. Schreiner  
Serum ultrafiltration for the elimination  
of endogenous interfering substances  
in creatinine determination.  
J.Clin.Chem.Clin.Biochem. 26, 523-525  
(1988)

R. H. Christenson, S. D. Studenberg,  
S. Beck-Davis and F. A. Sedor  
Digoxin-like immunoreactivity  
eliminated from serum by centrifugal  
ultrafiltration before fluorescence  
polarization immunoassay of digoxin.  
Clinical Chemistry 33, 606-608 (1987)

## Vivaspin 4



### 1–4 ml samples

Vivaspin 4 ml concentrators are disposable ultrafiltration devices for the concentration of biological samples. Maximum initial sample volumes range from 1 ml to 4 ml. They can be effectively used in either swing bucket or fixed angle rotors accepting 15 ml centrifuge tubes.

The patented vertical membrane design and thin channel filtration chamber (US 5,647,990) minimises membrane fouling and provides high speed concentrations, even with particle laden solutions.

Vivaspin 4 is available with the high flux polyethersulfone membrane range which is recommended for most solutions.

### Technical specifications Vivaspin 4

Concentrator capacity	Swing bucket rotor	4 ml
	Fixed angle rotor	4 ml
Dimensions	Total length	122 mm
	Width	17 mm
	Active membrane area	2.0 cm <sup>2</sup>
	Hold-up volume of membrane	< 10 µl
	Dead stop volume	20 µl
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polypropylene
	Concentrator cap	Polycarbonate
	Membrane	Polyethersulfone

### Equipment required Vivaspin 4

#### Centrifuge

Rotor type	Swing bucket	Fixed angle
Minimum rotor angle	–	25°
Rotor cavity	To fit 15 ml (17 mm) conical bottom tubes	To fit 15 ml (17 mm) conical bottom tubes
Maximum speed	4,000 g	10,000 g*

#### Concentrate recovery

Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type

\* Please note, devices with membrane MWCO >100 kDa need to be processed at lower g forces. See data sheets for details.

**Performance characteristics**

	<b>Time to concentrate up to 30x [min.] at 20°C and solute recovery %</b>	
	Min.	Rec.
Rotor	Fixed angle	
Centrifugal force	5,000 g	
Start volume	4 ml	
BSA 1.0 mg/ml (66,000 MW)		
5,000 MWCO PES	15	96%
10,000 MWCO PES	10	96%
30,000 MWCO PES	10	95%
IgG 0.25 mg/ml (160,000 MW)		
30,000 MWCO PES	10	95%
50,000 MWCO PES	10	95%
100,000 MWCO PES	10	95%

**Ordering information**

<b>Vivaspin 4 Polyethersulfone</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
5,000 MWCO	25	VS0413	14-558-452
5,000 MWCO	100	VS0414	14-558-453
10,000 MWCO	25	VS0403	14-558-450
10,000 MWCO	100	VS0404	14-558-451
30,000 MWCO	25	VS0423	14-558-454
30,000 MWCO	100	VS0424	14-558-455
50,000 MWCO	25	VS0433	14-558-456
50,000 MWCO	100	VS0434	14-558-457
100,000 MWCO	25	VS0443	14-558-458
100,000 MWCO	100	VS0444	14-558-459
0.2 µm	25	VS0473	14-558-460
0.2 µm	100	VS0474	14-558-461
Starter pack (5 of each 5 k, 10 k, 30 k, 50 k, 100 k)	25	VS04S3	14-558-462

## Vivaspin 6

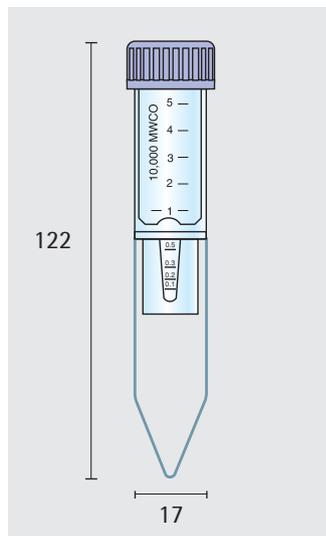


### 2–6 ml samples

Vivaspin 6 ml concentrators have been developed to offer increased volume flexibility and performance.

Vivaspin 6 can process an impressive 6 ml in either swing bucket or fixed angle rotors accepting standard 15 ml conical bottom test tubes.

The Vivaspin 6 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 concentrator and the modified dead stop pocket further simplifies direct pipette recovery of the final concentrate.



### Technical specifications Vivaspin 6

Concentrator capacity	Swing bucket rotor	6 ml
	Fixed angle rotor	6 ml
Dimensions	Total length	122 mm
	Width	17 mm
	Active membrane area	2.5 cm <sup>2</sup>
	Hold-up volume of membrane	<10 µl
	Dead stop volume	30 µl
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polycarbonate
	Concentrator cap	Polypropylene
	Membrane	Polyethersulfone

### Equipment required Vivaspin 6

#### Centrifuge

Rotor type	Swing bucket	Fixed angle
Minimum rotor angle	–	25°
Rotor cavity	To fit 15 ml (17 mm) conical bottom tubes	To fit 15 ml (17 mm) conical bottom tubes
Maximum speed	4,000 g	10,000 g*

#### Concentrate recovery

Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type

\* Please note, devices with membrane MWCO >100 kDa need to be processed at lower g forces. See data sheets for details.

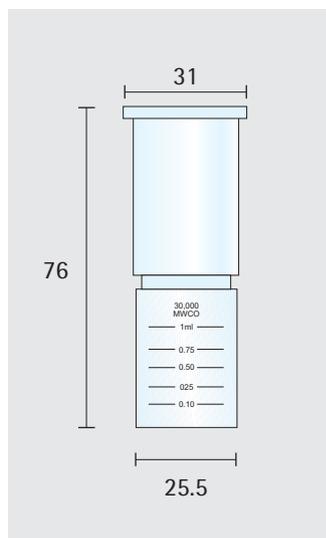
**Performance characteristics**

	Time to concentrate up to 30x [min.] at 20°C and solute recovery %			
	Swing bucket		25° Fixed angle	
Rotor	3,000 g		7,500 g	
Centrifugal force	6 ml		6 ml	
Start volume	Min.	Rec.	Min.	Rec.
Cytochrome c 0.25 mg/ml (12,400 MW) 5,000 MWCO PES	-	-	90	97%
BSA 1.0 mg/ml (66,000 MW) 5,000 MWCO PES	20	98%	12	98%
10,000 MWCO PES	13	98%	10	98%
30,000 MWCO PES	12	98%	9	97%
IgG 0.25 mg/ml (160,000 MW) 30,000 MWCO PES	18	96%	15	95%
50,000 MWCO PES	17	96%	14	95%
100,000 MWCO PES	15	91%	12	91%
Latex beads 0.004% in DMEM + 10% FCS (0.055 µm) 300,000 MWCO PES	-	-	25	99%
Latex beads 0.004% in DMEM + 10% FCS (0.24 µm) 1,000,000 MWCO PES	-	-	4	99%
Yeast 1.0 mg/ml ( <i>S. Cerevisiae</i> ) 0.2 µm PES	4	97%	3	97%

**Ordering information**

Vivaspin 6 Polyethersulfone	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
3,000 MWCO	25	VS0691	14-558-479
3,000 MWCO	100	VS0692	14-558-480
5,000 MWCO	25	VS0611	14-558-465
5,000 MWCO	100	VS0612	14-558-466
10,000 MWCO	25	VS0601	14-558-463
10,000 MWCO	100	VS0602	14-558-464
30,000 MWCO	25	VS0621	14-558-467
30,000 MWCO	100	VS0622	14-558-468
50,000 MWCO	25	VS0631	14-558-469
50,000 MWCO	100	VS0632	14-558-470
100,000 MWCO	25	VS0641	14-558-471
100,000 MWCO	100	VS0642	14-558-472
300,000 MWCO	25	VS0651	14-558-473
300,000 MWCO	100	VS0652	14-558-474
1,000,000 MWCO	25	VS0661	14-558-475
1,000,000 MWCO	100	VS0662	14-558-476
0.2 µm	25	VS0671	14-558-477
0.2 µm	100	VS0672	14-558-478
Starter pack (5 of each 5 k, 10 k, 30 k, 50 k, 100 k)	25	VS06S1	14-558-481

## Vivaspin 15



### 2–15 ml samples

The Vivaspin 15 concentrator is a disposable ultrafiltration device for use in swing bucket centrifuges accommodating 50 ml tubes. Vivaspin 15 is used for the concentration of biological samples in the 2–15 ml range. The innovative design (US Patent no. 5,647,990, second patent pending), simplicity, speed and exceptional concentrate recoveries are the main features of the concentrator.

In a single spin, 15 ml solutions can be concentrated up to 300x. Samples can be typically concentrated in 10–30 minutes with macromolecular recoveries in excess of 95%. The longitudinal membrane location and adjacent thin channel, provide optimum cross flow conditions even for particle laden solutions, the centrifugal force pulling particles and solids away from the membrane to the bottom of the device. Macromolecules collect in an impermeable 50  $\mu$ l concentrate pocket integrally moulded below the membrane surface, thereby eliminating the risk of filtration to dryness.

### Technical specifications Vivaspin 15

Concentrator capacity	Swing bucket rotor	15 ml
	Fixed angle rotor	8 ml
Dimensions	Total length	76 mm
	Width	25.5 mm
	Active membrane area	4 cm <sup>2</sup>
	Hold up volume of membrane	<20 $\mu$ l
	Dead stop volume	50 $\mu$ l
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polypropylene
	Concentrator cap	Polycarbonate
	Membrane	Polyethersulfone

### Equipment required Vivaspin 15

<b>Centrifuge</b>		
Rotor type	Swing bucket	Fixed angle
Minimum rotor angle	–	25°
Rotor cavity	To fit 50 ml (17 mm) conical bottom tubes	To fit 50 ml (17 mm) conical bottom tubes
Maximum speed	3,000 g*	3,000 g
<b>Concentrate recovery</b>		
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type

\* Please note, devices with membrane MWCO >100 kDa need to be processed at lower g forces. See data sheets for details.

**Performance characteristics**

	<b>Time to concentrate up to 30x [min.] at 20°C and solute recovery %</b>	
	Min.	Rec.
Rotor	Fixed angle	
Centrifugal force	2,000 g	
Start volume	15 ml	
<b>BSA 1 mg/ml (66,000 MW)</b>		
5,000 MWCO	40	97%
10,000 MWCO	25	97%
30,000 MWCO	25	96%
50,000 MWCO	25	96%
100,000 MWCO	15	70%
<b>Cytochrome c 0.25 mg/ml (12,400 MW)</b>		
5,000 MWCO	55	97%
10,000 MWCO	45	95%
30,000 MWCO	45	59%
50,000 MWCO	45	40%
100,000MWCO	20	16%
<b>IgG 0.25 mg/ml (160,000 MW)</b>		
30,000 MWCO	30	94%
50,000 MWCO	30	94%
100,000 MWCO	30	90%
<b>Yeast 1.0 mg/ml (S. Cerevisiae)</b>		
100,000 MWCO	15	98%
0.2 µm PES	7	95%

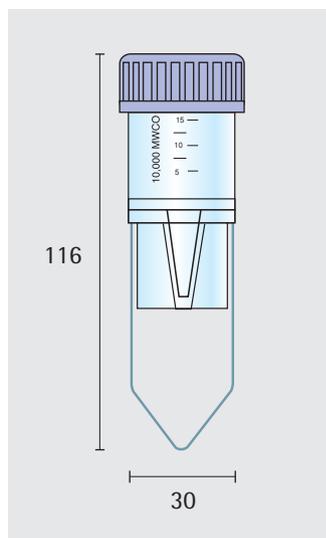
**Ordering information – Requires 50 ml centrifuge tubes**

<b>Vivaspin 15 Polyethersulfone</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
5,000 MWCO	10	VS1511	14-558-484
5,000 MWCO	40	VS1512	14-558-485
10,000 MWCO	10	VS1501	14-558-482
10,000 MWCO	40	VS1502	14-558-483
30,000 MWCO	10	VS1521	14-558-486
30,000 MWCO	40	VS1522	14-558-487
50,000 MWCO	10	VS1531	14-558-488
50,000 MWCO	40	VS1532	14-558-489
100,000 MWCO	10	VS1541	14-558-490
100,000 MWCO	40	VS1542	14-558-491
Starter pack (2 of each 5 k, 10 k, 30 k, 50 k, 100 k)	10	VS15S1	14-558-500

**Accessories**

Conical bottom 50 ml tubes and lids	100	VSA001	14-558-532
Conical bottom 50 ml tubes and lids	40	VSA002	■■■■

## Vivaspin 15R



### 2–15 ml samples

Vivaspin 15R is the latest member of the Vivaspin product family with all the unique features of Sartorius Stedim Biotech concentrators including a patented vertical membrane and a dead stop. Vivaspin 15R is targeting the volume segment 2 to 15 ml with a modified regenerated cellulose membrane; Hydrosart®. This membrane is ideal where extremely high recovery with very low adsorption is needed, for example in applications such as desalting and concentration of Ig fractions.

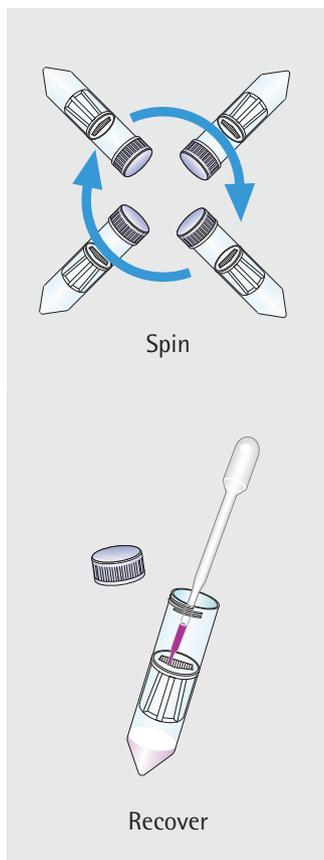
- Ultimate recovery at low adsorption (95-98%)
- Extremely short concentration time (30x in 15 min.)
- Convenient application protocol with easy handling
- Easy scale-up to Vivaflow 200 with Hydrosart® membrane for volumes up to 5 litres
- Very small hold up volume (< 20 µl)

### Technical specifications Vivaspin 15R

Concentrator capacity	Swing bucket rotor	15 ml
	Fixed angle rotor	12.5 ml
Dimensions	Total length	116 mm
	Width	30 mm
	Active membrane area	3.9 cm <sup>2</sup>
	Hold up volume membrane	< 20 µl
	Dead stop volume	30 µl
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polypropylene
	Concentrator cap	Polycarbonate
	Membrane	Hydrosart

### Equipment required Vivaspin 15R

<b>Centrifuge</b>		
Rotor type	Swing bucket	Fixed angle
Minimum rotor angle	–	25°
Rotor cavity	To fit 50 ml (30 mm) conical bottom tubes	To fit 50 ml (30 mm) conical bottom tubes
Maximum speed	3,000 g	6,000 g
<b>Concentrate recovery</b>		
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type



### Performance characteristics

	Time to concentrate up to 30x [min.] at 20°C and solute recovery %			
	Swing bucket		25° Fixed angle	
Rotor	3,000 g		6,000 g	
Centrifugal force	15 ml		12.5 ml	
Start volume	Min.	Rec.	Min.	Rec.
Aprotinin 0.1 mg/ml* (6,500 MW) 5,000 MWCO	47	95%	45	95%
Cytochrome c 0.25 mg/ml* (12,400 MW) 5,000 MWCO	45	96%	45	96%
10,000 MWCO	25	94%	18	94%
$\alpha$ -chymotrypsin 0.25 mg/ml* (25,000 MW) 5,000 MWCO	50	98%	45	98%
10,000 MWCO	25	98%	18	98%
Ovalbumin 1.0 mg/ml* (45,000 MW) 10,000 MWCO	20	98%	14	98%
30,000 MWCO	15	94%	12	94%
BSA 1.0 mg/ml* (66,000 MW) 30,000 MWCO	18	98%	15	98%
IgG 0.1 mg/ml* in DMEM (160,000 MW) 30,000 MWCO	30	98%	25	96%

\* proteins other than IgG made up in 50 mM potassium phosphate, 150 mM sodium chloride, pH 7.4

### Ordering information

Vivaspin 15R Hydrosart	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
2,000 MWCO	12	VS15RH91	14-558-498
2,000 MWCO	48	VS15RH92	14-558-499
5,000 MWCO	12	VS15RH11	14-558-494
5,000 MWCO	48	VS15RH12	14-558-495
10,000 MWCO	12	VS15RH01	14-558-492
10,000 MWCO	48	VS15RH02	14-558-493
30,000 MWCO	12	VS15RH21	14-558-496
30,000 MWCO	48	VS15RH22	14-558-497

## Vivaspin 20



### 5–20 ml samples

Vivaspin 20 ml centrifugal concentrators have been developed to offer increased volume flexibility and performance.

Vivaspin 20 handles up to 20 ml in swing bucket centrifuges and 14 ml in 25° fixed angle rotors accepting 50 ml centrifuge tubes.



Featuring twin vertical membranes for unparalleled filtration speeds the Vivaspin 20 can achieve 100x plus concentrations.

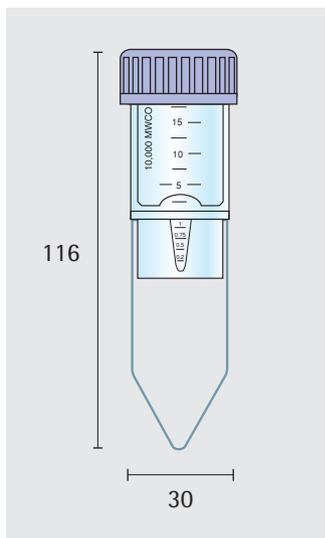
Remaining volume is easy to read off the printed scale on the side of the concentrator and the modified dead stop pocket further simplifies direct pipette recovery of the final concentrate.

### More process flexibility

Vivaspin 20 is available with unique accessories and operating methods that are designed to provide more process flexibility and further time saving.

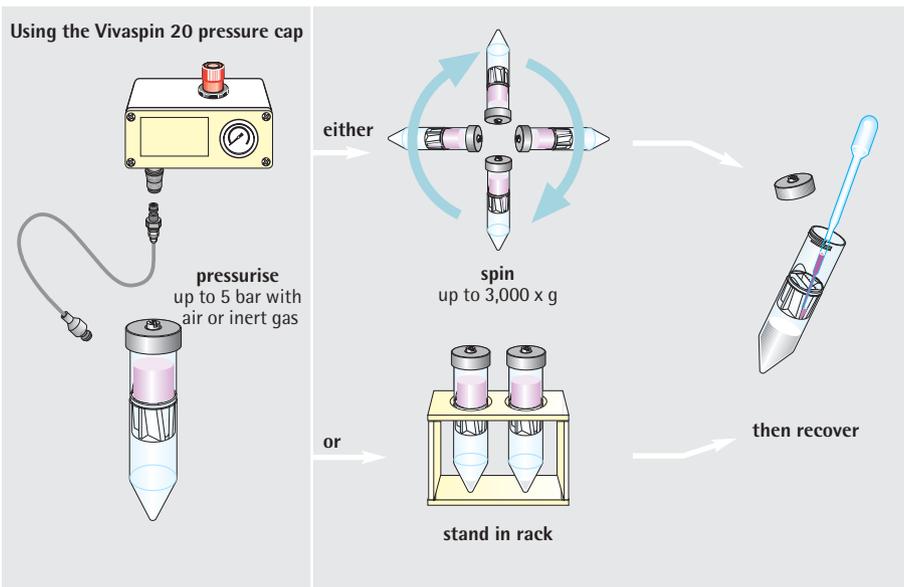
### Gas pressure filtration

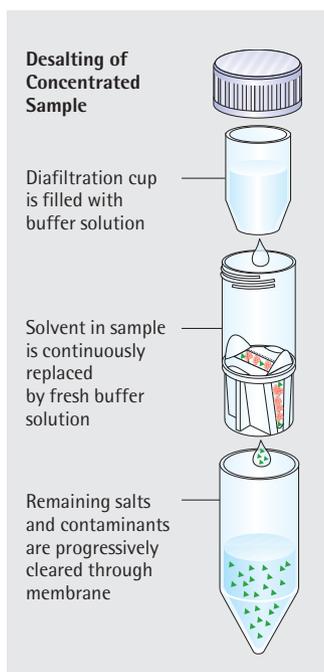
When an appropriate centrifuge is unavailable, or for single sample processing, Vivaspin 20 can be filled with up to 15 ml and then pressurised for bench top concentration. For even faster processing, gas pressure can be combined with centrifugal force. "Pressure-fugation" is particularly suitable for difficult or viscous samples such as serum, or when using a low process temperature which reduces filtration speed, and generally when minimum process time is essential.



### Technical specifications Vivaspin 20

Concentrator capacity	Swing bucket rotor	20 ml
	Fixed angle rotor	14 ml
	With pressure head	15 ml
Dimensions	Total length	116 mm
		125 mm with pressure head
	Width	30 mm
	Active membrane area	6.0 cm <sup>2</sup>
	Hold up volume of membrane	< 20 µl
Materials of construction	Dead stop volume	50 µl
	Body	Polycarbonate
	Filtrate vessel	Polycarbonate
	Concentrator cap	Polypropylene
	Pressure head	Acetal   aluminium
	Membrane	Polyethersulfone





### Desalting with Vivaspin 20

In this procedure following concentration, a diafiltration cup is filled with buffer and then spun one time to achieve 98% salt removal. This compares to the need for two spins to achieve the same result with the traditional refill and re-spin procedure.

The improved performance is due to the constant washing action of the buffer solution in the diafiltration cup as it replaces solvent and salts as they pass through the ultrafiltration membrane.

### Equipment required Vivaspin 20

#### Centrifuge

Rotor type	Swing bucket	Fixed angle
Minimum rotor angle	–	25°
Rotor cavity	To fit 50 ml (30 mm) conical bottom tubes	To fit 50 ml (30 mm) conical bottom tubes
Maximum speed	5,000 g*	8,000 g*

#### Optional pressure accessories

Air pressure controller (APC) complete with pressure gauge, regulator, over-pressure safety valve, female connector to Sartorius Stedim Biotech pressure products and 1 m extension line (4 mm pneumatic tubing) with male and female connectors and 1 m of 6 mm inlet tubing

Charge valve	VCA005	14-558-290
VS20 pressure head	VCA200	14-558-299

<b>Sartorius Stedim</b>	<b>Fisher Scientific</b>
<b>Order No.</b>	<b>Order No.</b>
VCA002	14-558-288

#### Concentrate recovery

Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type

\* Please note, devices with membrane MWCO >100 kDa need to be processed at lower g forces. See data sheets for details.

#### Performance characteristics

Mode	Time to concentrate up to 30x [min.] at 20°C and solute recovery %							
	Centrifuge		Centrifuge		Bench top		Press-fuge	
Rotor	Swing bucket		25° Fixed angle		Pressure		Swing bucket	
Centrifugal speed   pressure	3,000 g		6,000 g		4 bar		3,000 g + 4 bar	
Start volume	20 ml		14 ml		10 ml		10 ml	
	Min.	Rec.	Min.	Rec.	Min.	Rec.	Min.	Rec.
Cytochrome c 0.25 mg/ml (12,400 MW)								
3,000 MWCO PES	110	97%	180	96%	60	96%	–	–
BSA 1.0 mg/ml (66,000 MW)								
5,000 MWCO PES	23	99%	29	99%	50	98%	14	98%
10,000 MWCO PES	16	98%	17	98%	32	97%	8	97%
30,000 MWCO PES	13	98%	15	98%	32	97%	8	97%
IgG 0.25 mg/ml (160,000 MW)								
30,000 MWCO PES	27	97%	20	95%	46	94%	13	97%
50,000 MWCO PES	27	96%	22	95%	46	93%	13	96%
100,000 MWCO PES	25	91%	20	90%	42	88%	12	94%
Latex beads 0.004% in DMEM +10% FCS (0.055 µm)								
300,000 MWCO PES	20	99%	35	99%	10	99%	–	–
Latex beads 0.004% in DMEM +10% FCS (0.24 µm)								
1,000,000 MWCO PES	4	99%	12	99%	4	99%	–	–
Yeast 1.0 mg/ml ( <i>S. Cerevisiae</i> )								
0.2 µm PES	15	95%	5	95%	20	95%	2	95%

**Ordering information**

<b>Vivaspin 20 Polyethersulfone</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
3,000 MWCO	12	VS2091	14-558-517
3,000 MWCO	48	VS2092	14-558-518
5,000 MWCO	12	VS2011	14-558-503
5,000 MWCO	48	VS2012	14-558-504
10,000 MWCO	12	VS2001	14-558-501
10,000 MWCO	48	VS2002	14-558-502
30,000 MWCO	12	VS2021	14-558-505
30,000 MWCO	48	VS2022	14-558-506
50,000 MWCO	12	VS2031	14-558-507
50,000 MWCO	48	VS2032	14-558-508
100,000 MWCO	12	VS2041	14-558-509
100,000 MWCO	48	VS2042	14-558-510
300,000 MWCO	12	VS2051	14-558-511
300,000 MWCO	48	VS2052	14-558-512
1,000,000 MWCO	12	VS2061	14-558-513
1,000,000 MWCO	48	VS2062	14-558-514
0.2 µm	12	VS2071	14-558-515
0.2 µm	48	VS2072	14-558-516
Starter pack (2 of each 5 k, 10 k, 30 k, 50 k, 100 k, 0.2 µm)	12	VS20S1	14-558-519

**Vivaspin 20 accessories**

Air pressure controller (APC)	1	VCA002	14-558-288
Charge valve for pressure head	1	VCA005	14-558-290
Diafiltration cups	12	VSA005	14-558-534
Female connector	1	VCA010	14-558-294
Male connector	1	VCA011	14-558-295
4 mm OD pneumatic tube (3 m)	1	VCA012	14-558-296
Vivaspin 20 pressure head	1	VCA200	14-558-299

## Vivaclear Centrifugal Filters



Vivaclear centrifugal filters are disposable microfiltration devices for the fast and reliable clarification | filtration of biological samples in the range 100 µl to 500 µl. They can be used in fixed angle rotors accepting 2.2 ml centrifuge tubes.

### Product Features

- High-flux Polyethersulphone membrane
- 0.8 µm pore size
- Low hold up volume (<5 µl)
- Fast and reproducible performance

### Applications

- Clarification of samples before loading onto Vivapure protein purification spin columns
- Removal of particles and particulates
- Filtration of plasma and serum
- Filtration of cells or cell debris

### Technical specifications

Rotor	40–45° Fixed angle rotor 500 µl		
Pore size	0.8 µm		
Dimensions	Total length	43 mm	
	Filtrate collection tube diameter	11 mm	
	Active membrane area	0.34 cm <sup>2</sup>	
	Hold-up volume, membrane plus support	< 5 µl	
	Maximum RCF	2,000 × g	
Materials of construction	Body	Polypropylene	
	Membrane	Polyethersulphone	
	Filtrate collection tube	Polypropylene	
Ordering information	Pack size	<b>Sartorius Stedim</b>	<b>Fisher Scientific</b>
		<b>Order No.</b>	<b>Order No.</b>
Vivaclear Mini 0.8 µm PES	100	VK01P042	14-558-342

## Vivacell 70



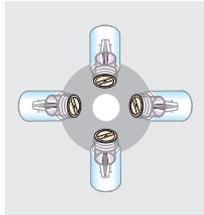
### 10–70 ml samples

Vivacell 70 combines the ease of use of centrifugal devices with the flexibility and control provided by pressurised ultrafiltration cells. Vivacell 70 is inexpensive, quick and easy to assemble, requires no tubing connections or stirring mechanisms and can be adapted to equipment availability or to specific user preferences.

For convenience, simply spin in a large capacity centrifuge (rotors accepting 250 ml bottles). For highest speeds particularly with difficult samples, pressurise the device with air or inert gas before centrifuging.

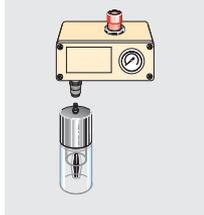
For more process control or for single samples, combine gas pressure with a gentle orbital shake, or you can even pressurise and then leave standing on a bench top or in a refrigerator for highest simplicity with minimum equipment requirements.

The longitudinal membrane inhibits fouling, whilst the built-in dead stop will hinder further concentration when residual volume drops below 150  $\mu$ l.



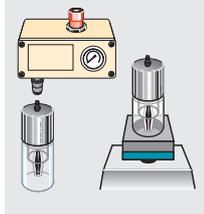
### Centrifuge

- Process convenience
- Low shear, no foaming
- Less visual control



### Pressurise

- Simplicity and highest process control
- Ideal for refrigerated use
- Slower concentrations



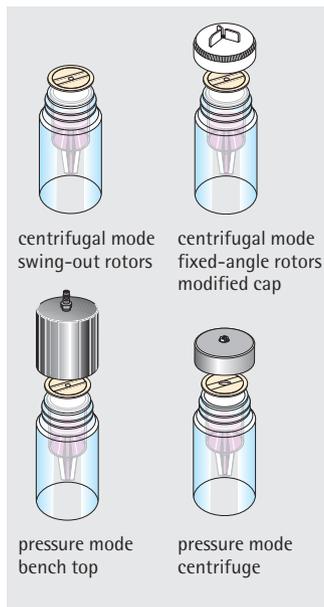
### Pressure-shake

- Speed and process control
- Ideal for single samples
- If left unattended can concentrate to dryness



### Pressure-fuge

- Fastest processing
- Ideal with low MWCO or with difficult solutions
- Less visual control

**Total process flexibility**

**Technical specifications Vivacell 70**

Concentrator capacity	Swing bucket rotor	70 ml
	Fixed angle rotor	50 ml
	With pressure head	70 ml
	With pressure-fuge head	50 ml
Dimensions	Total length	119 mm standard centrifugal 185 mm with pressure head 125 mm with pressure fuge head
	Width	62 mm
	Active membrane area	20 cm <sup>2</sup>
	Hold up volume of membrane	< 200 µl
	Dead stop volume	150 µl
	Operating requirements	Rotor type
Minimum rotor angle		25°
Rotor cavity		To fit 250 ml (62 mm) centrifuge bottles
Maximum speed		1,000 g
Maximum pressure		5 bar (75 psi)
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polycarbonate
	Concentrator cap	Santoprene
	Pressure head   pressure fuge head	Acetal
	Membrane	Polyethersulfone

**Performance characteristics**

50 ml Start volume	Time to concentrate up to 30x [min.] at 20°C				Solute recovery %
	In centrifuge 1,000 g		As pressure cell 4 bar (60 psi) pressure		
	No pressure	3 bar pressure	No agitation	Orbital shake	
BSA 1.0 mg/ml (66,000 MW)					
5,000 MWCO PES	37	18	50	25	96%
10,000 MWCO PES	25	15	45	20	96%
30,000 MWCO PES	22	13	45	20	93%
IgG 0.25 mg/ml (160,000 MW)					
50,000 MWCO PES	25	15	85	20	94%
100,000 MWCO PES	15	11	90	18	90%

**Ordering information**

<b>Vivacell 70 Polyethersulfone – concentrator bodies with polycarbonate filtrate bottles</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
5,000 MWCO	2	VS6011	14-558-522
10,000 MWCO	2	VS6001	14-558-520
30,000 MWCO	2	VS6021	14-558-524
50,000 MWCO	2	VS6031	14-558-526
100,000 MWCO	2	VS6041	14-558-528
0.2 µm	2	VS6071	14-558-530

**Vivacell 70 Polyethersulfone –  
concentrator body only**

5,000 MWCO	10	VS6012	14-558-523
10,000 MWCO	10	VS6002	14-558-521
30,000 MWCO	10	VS6022	14-558-525
50,000 MWCO	10	VS6032	14-558-527
100,000 MWCO	10	VS6042	14-558-529
0.2 µm	10	VS6072	14-558-531

**Vivacell 70 accessories**

Air pressure controller (APC) complete with pressure gauge, regulator, over-pressure safety valve, female connector to Sartorius Stedim Biotech pressure products and 1 m extension line (4 mm pneumatic tubing) with male and female connectors and 1 m of 6 mm inlet tubing	1	VCA002	14-558-288
250 ml centrifuge bottle – standard caps	4	VSA003	14-558-533
Modified caps for use in fixed angle rotors with 250 ml centrifuge bottles	2	VCA004	14-558-289
Charge valve for pressure-fuge head	1	VCA005	14-558-290
Replacement seals for pressure-fuge head (VCA701   14-558-302)	10	VCA007	14-558-291
Female connector	1	VCA010	14-558-294
Male connector	1	VCA011	14-558-295
4 mm pneumatic tubing (3 m)	1	VCA012	14-558-296
Vivacell 70 pressure head with reservoir and filtrate bottle (bench top use)	1	VCA700	14-558-301
Vivacell 70 pressure-fuge head (for use in centrifuge)	2	VCA701	14-558-302

## Vivacell 100



### 20–100 ml Samples

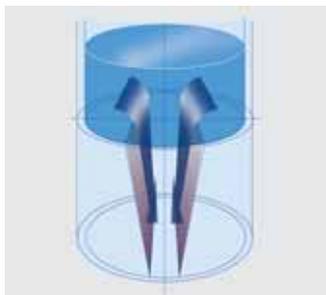
Vivacell 100 is the latest member of the Vivacell family and bridges the volume range between the Vivacell 70 and the Vivacell 250.

The patented vertical membrane design allows highest performance and unmatched flexibility.



Vivacell 100 is a unique and innovative concentrator for volumes from 20 ml to 100 ml, which utilizes pressure, centrifuge or pressure-shake to rapidly concentrate even samples with very high particle loading.

Vivacell 100 is designed for centrifugal concentration of samples up to 100 ml which makes it the largest centrifugal unit available. At the same time, the new construction design allows for maximum centrifugal force of 4,000x g to be used for even faster concentration.



### Vivacell 100 utilizes:

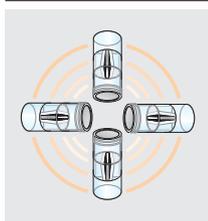
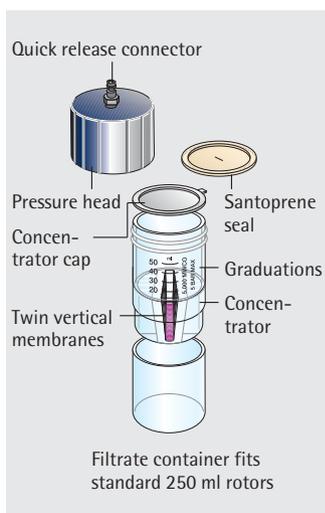
- Pressure
- Centrifuge
- Pressure-shake

Like the smaller Vivacell 70 unit, Vivacell 100, when used as a centrifugal device, fits only into swing bucket rotors accepting 250 ml bottles.

Vivacell 100 units can also be used for single or extremely sensitive samples in the pressurized mode only and left on the bench or placed on a laboratory shaker for faster concentration. It can also be kept in a pressurized mode in the refrigerator. Handling is made easy by use of quick connectors. In whichever mode Vivacell 100 is used, the vertical membrane design inhibits membrane fouling while the built-in dead stop impedes concentration to dryness and loss of sample.

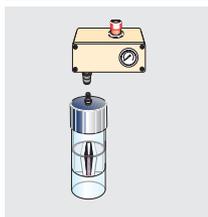
### Technical specifications Vivacell 100

Concentrator capacity	Swing bucket rotor	90 ml
	With pressure head	98 ml
Dimensions	Total length	123 mm centrifugal 197 mm with pressure head
	Width	62 mm
	Active membrane area	23.5 cm <sup>2</sup>
	Hold up volume of membrane	< 250 µl
	Dead stop volume	350 µl
Operating requirements	Rotor type	Swing bucket
	Rotor cavity	To fit 250 ml (62 mm) centrifuge bottles (maximum cavity depth 105 mm)
	Maximum speed	2,000 g
	Maximum pressure	5 bar (75 psi)
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polycarbonate
	Concentrator cap	Santoprene
	Pressure head	Acetal
	Membrane	Polyethersulfone



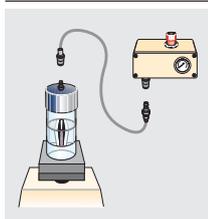
### Centrifuge

- Process convenience
- Low shear, no foaming
- Less visual control



### Pressure

- Simplicity and highest process control
- Ideal for refrigerated use
- Slower concentrations



### Pressure-shake

- Speed and process control
- Ideal for single samples

## Performance characteristics

### Time to concentrate up to 30x [min.] at 20°C

90 ml start volume	In centrifuge 2,000 g swing-out rotor	As pressure cell 4 bar (60 psi)		Solute recovery %
		No agitation	Orbital shake	
<b>BSA 1.0 mg/ml (66,000 MW)</b>				
5,000 MWCO PES	22	75	25	96%
10,000 MWCO PES	16	60	20	96%
30,000 MWCO PES	16	60	20	94%
<b>IgG 0.25 mg/ml (160,000 MW)</b>				
50,000 MWCO PES	20	70	30	94%
100,000 MWCO PES	20	85	30	90%
<b>Latex beads 0.004% in DMEM + 10% FCS (0.055 µm)</b>				
300,000 MWCO PES	35	-	120	99%
<b>Latex beads 0.004% in DMEM + 10% FCS (0.24 µm)</b>				
1,000,000 MWCO* PES	4	5	4	99%

\* 2,000 g in centrifuge, 2 bar (29 psi) pressure

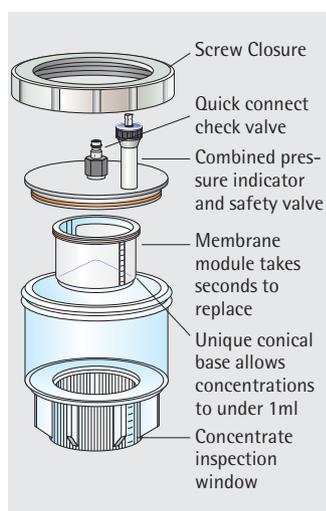
**Ordering information**

<b>Vivacell 100 Polyethersulfone With Polypropylene concentrator cap</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
5,000 MWCO	2	VC1011	14-558-267
5,000 MWCO	10	VC1012	14-558-268
10,000 MWCO	2	VC1001	14-558-265
10,000 MWCO	10	VC1002	14-558-266
30,000 MWCO	2	VC1021	14-558-269
30,000 MWCO	10	VC1022	14-558-270
50,000 MWCO	2	VC1031	14-558-271
50,000 MWCO	10	VC1032	14-558-272
100,000 MWCO	2	VC1041	14-558-273
100,000 MWCO	10	VC1042	14-558-274
300,000 MWCO	2	VC1051	14-558-275
300,000 MWCO	10	VC1052	14-558-276
1,000,000 MWCO	2	VC1061	14-558-277
1,000,000 MWCO	10	VC1062	14-558-278
0.2 µm	2	VC1071	14-558-279
0.2 µm	10	VC1072	14-558-280

**Accessories**

	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
Air pressure controller (APC) complete with pressure gauge, regulator, over-pressure safety valve, female connector, 1 m extension line (4 mm pressure tubing) with male and female connectors and 1 m of 6 mm inlet tubing	1	VCA002	14-558-288
Plastic pipettes	100	VPA005	14-558-393
Female connector	1	VCA010	14-558-294
Male connector	1	VCA011	14-558-295
4 mm pressure tubing (3 m)	1	VCA012	14-558-296
Santoprene replacement seals	10	VCA014	14-558-297
Vivacell 100 pressure head with replacement seals (5)	1	VCA800	14-558-303

## Vivacell 250



### 50–250 ml samples

The Vivacell 250 is a totally new concept for the concentration of larger biological samples. This product offers numerous advantages when compared to stirred cells.

- One size handles a volume range from under 50 ml to 250 ml.
- Use free standing on a bench top or in a refrigerator for maximum simplicity, or use on laboratory shaker for fastest concentrations.
- The unique conical dead stop built into the bottom of the membrane insert allows concentrations to under 1 ml.
- The gentle vortex action controls membrane polarisation whilst greatly reducing the shear effects typical of stirring mechanisms.
- Set up or membrane replacement takes just a few seconds. Quick connect fittings and simple screw closure further enhance ease of use.

Unique membrane module takes seconds to replace. Concentrate can be easily monitored through the graduated inspection window.

### Technical specifications Vivacell 250

Concentrator capacity	250 ml	
Max pressure	4 bar (60 psi)	
Dimensions	Width	116 mm
	Height (incl. pressure indicator)	235 mm
	Active membrane area	40 cm <sup>2</sup>
	Hold-up vol. memb. & support	< 200 µl
	Dead stop volume	600 µl
Materials of construction	Screw closure	Acetal
	Pressure head	Acetal
	Quick release connector	Acetal
	Concentrator body   sleeve	Polycarbonate
	Filtrate container	Polycarbonate

### Performance characteristics

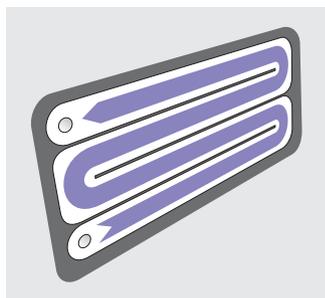
Time to concentrate up to 20x [min.] at 20°C 4 bar pressure

	100 ml start volume			250 ml start volume		
	Orbital shake	Free standing	Solute recovery %	Orbital shake	Free standing	Solute recovery %
BSA 1.0 mg/ml (66,000 MW)						
5,000 MWCO PES	19	70	98%	40	140	99%
10,000 MWCO PES	12	45	97%	28	100	98%
30,000 MWCO PES	12	45	96%	28	100	98%
γ Globulins 0.25 mg/ml (160,000 MW)						
30,000 MWCO PES	25	120	96%	55	240	98%
50,000 MWCO PES	25	120	94%	55	240	98%
100,000 MWCO PES	25	120	96%	58	240	98%

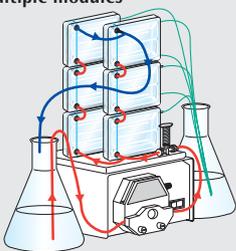
### Ordering information

Vivacell 250	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
Vivacell 250 complete with pressure head, pressure indicator   over-pressure release valve, quick release connection to APC, 2 sample reservoirs, filtrate container & insert tool	1	VCA250	14-558-300
<b>Vivacell 250 Polyethersulfone inserts</b>			
5,000 MWCO	5	VC2511	14-558-282
10,000 MWCO	5	VC2501	14-558-281
30,000 MWCO	5	VC2521	14-558-283
50,000 MWCO	5	VC2531	14-558-284
100,000 MWCO	5	VC2541	14-558-285
0.2 µm	5	VC2571	14-558-286
Starter kit (1 of each 5 k, 10 k, 30 k, 50 k, 100 k)	5	VC25S1	14-558-287
<b>Accessories</b>			
Air pressure controller (APC) complete with pressure gauge, regulator, over-pressure safety valve, female connector to Sartorius Stedim Biotech pressure products and 1 m extension line (4 mm pneumatic tubing) with male and female connector and 1 m of 6 mm inlet tubing	1	VCA002	14-558-288
Replacement pressure indicator   over pressure relief valve	1	VCA008	14-558-292
Vivacell 250 maintenance kit (includes one sample reservoir and filtrate container, and "O" ring seals for pressure head)	1	VCA009	14-558-293
Female connector	1	VCA010	14-558-294
Male connector	1	VCA011	14-558-295
4 mm OD pressure tubing (3 m)	1	VCA012	14-558-296
Replacement pressure head & screw closure	1	VCA015	14-558-298

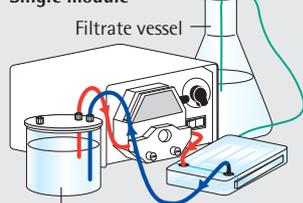
## Vivaflow 50



Multiple modules



Single module



Sample | diafiltration reservoir  
(Sartorius Stedim Order No. VFA006 |  
Fisher Scientific Order No.  
14-558-327)

### 100 ml to 5 litres

The novel Vivaflow 50 system (patents pending) provides a standard of ease of use, performance, flexibility and economy which is unrivalled by any laboratory or pilot scale filtration system on the market.

### Unique features

- Thin channel flip-flow recirculation path provides high cross flow velocities with minimum pump requirements.
- No need for pressure holders.
- Crystal clear for simple control of remaining hold up and membrane status.
- Unique Interlocking modules with series connectors for easy scale up.
- Disposable.

### Unique performance

- A single 50 cm<sup>2</sup> module will typically reduce 500 ml to less than 15 ml in under 50 minutes.
- Less than 10 ml minimum system recirculation for highest concentrations.
- Less than 500 µl non recoverable hold up volume.
- Near total recoveries achievable with a single 10 ml rinse.

Unique "flip-flow" thin channel flow path results in high turbulence and linear velocity for exceptional flux even at high concentrations

### Technical specifications Vivaflow 50

Dimensions	Overall L   H   W	107   84   25 mm
	Channel W   H	15 mm   0.3 mm
	Active membrane area	50 cm <sup>2</sup>
	Hold up volume (module)	1.5 ml
	Minimum recirculation volume	< 10 ml
Operating conditions	Non recoverable hold-up	< 0.5 ml
	Pump flow	200-400 ml/min
	Maximum pressure	3 bar (45 psi)
Materials of construction	Maximum temperature	60°C
	Main housing	Polycarbonate
	Flow channel	TPX (PMP)
	Membrane support	TPX (PMP)
	Seals and O rings	Silicone
	Pressure indicator	Polypropylene, SS spring
	Flow restrictor	Polypropylene
	Fittings	Nylon
	Tubing	PVC (medical grade)

### Performance characteristics

Time to concentrate up to 20x [min.] at 3 bar inlet pressure, 20°C

	Single device 250 ml start volume	Three devices 1 L start volume	Solute recovery %	
			Direct	10 ml rinse
BSA 1.0 mg/ml (66,000 MW)				
5,000 MWCO PES	34	49	96%	> 99%
10,000 MWCO PES	22	32	94%	> 99%
10,000 MWCO RC	38	55	96%	> 99%
30,000 MWCO PES	22	32	92%	99%
50,000 MWCO PES	20	29	92%	98%
γ Globulins 1.0 mg/ml (160,000 MW)				
100,000 MWCO PES	43	62	92%	98%
100,000 MWCO RC	40	58	92%	98%
Yeast 1.0 mg/ml ( <i>S.Cerevisiae</i> )				
0.2 µm PES	33	47	92%	98%



### Ordering information

Vivaflow 50 modules include filtrate tube, size 16 peristaltic tubing, flow restrictor and fittings	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
3,000 MWCO PES	2	VF05P9	14-558-312
5,000 MWCO PES	2	VF05P1	14-558-307
10,000 MWCO PES	2	VF05P0	14-558-306
30,000 MWCO PES	2	VF05P2	14-558-308
50,000 MWCO PES	2	VF05P3	14-558-309
100,000 MWCO PES	2	VF05P4	14-558-310
0.2 µm PES	2	VF05P7	14-558-311
10,000 MWCO RC	2	VF05C0	14-558-304
100,000 MWCO RC	2	VF05C4	14-558-305

### Vivaflow 50 complete system comprises:

Pump (115 V), Easy load pump head (size 16), tubing, 500 ml sample   diafiltration reservoir, module stand, pressure indicator, T connectors, series interconnectors	1	VFS504	14-558-341
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### Vivaflow 50 PVC tubing and fittings

Size 16 PVC pump tubing (3 metres, 3.2 × 1.6 mm)	VFA004	14-558-325
Flow restrictor set (2 × 0.4, 0.6, 0.8 mm)	VFA009	14-558-328
T connectors for running 2 stacks (2 pieces)	VFA030	14-558-333
Series interconnectors (6 pieces)	VFA031	14-558-334
Female luer fittings (10 pieces)	VFA032	14-558-335
VF50 tubing Kit (2 × 1 m size 16 PVC tubing with inlet fittings, 2 × 50 cm size 16 PVC tubing with 0.6 mm flow restrictors, 1 × series interconnector)	VFA034	14-558-336
Flow restrictor 0.6 mm (6 pieces)	VFA035	14-558-337

### VivaFlow 50 accessories

Masterflex economy drive variable speed peristaltic pump (115 V)	VFP002	14-558-339
500 ml sample and   or diafiltration reservoir	VFA006	14-558-327
Masterflex easy load pump head – size 16	VFA012	14-558-329
Vivaflow 50 stand	VFA016	14-558-331
Pressure indicator (1-3 bar)	VFA020	14-558-332

## Vivaflow 200



### 0.5 to 5 litres

Concentrate 250 ml to under 20 ml in just a few minutes or concentrate one litre 50 times in less than 30 minutes. Alternatively, use two Vivaflow 200's in parallel and concentrate 5 litres in under 75 minutes.

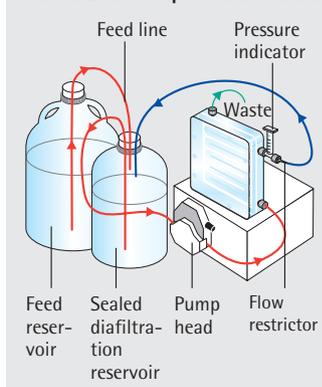
Near total sample recoveries can be expected with most solutions.

The economical standard package comes complete with tubing, pressure indicator, flow restrictor and high pressure pump tubing. All you need is a peristaltic pump capable of handling 6.4 mm OD (size 16) tubing. Should your pump head require larger tubing, link your own peristaltic tube up to the standard product, using the interconnector provided.

Two modules in parallel will concentrate 5 litres in under 75 minutes



Vivaflow 200 set-up for diafiltration



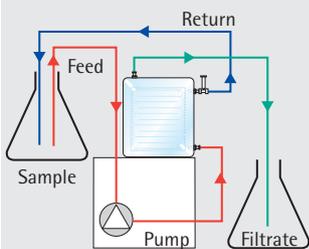
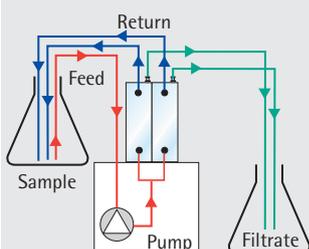
### Technical specifications Vivaflow 200

Dimensions	Overall L   H   W	126   138   38 mm
	Channel W   H	10 mm   0.4 mm
	Active membrane area	200 cm <sup>2</sup>
	Hold up volume (module)	5.3 ml
	Min. recirculation volume	< 20 ml
	Non recoverable hold-up	< 2 ml
Materials of construction	Main housing	Acrylic
	Flow channel	Acrylic
	Membrane support	Polypropylene
	Seals and O rings	Silicone
	Pressure indicator	Polypropylene, SS spring
	Flow restrictor	Polypropylene
	Fittings	Nylon
	Tubing	PVC (medical grade)
Operating conditions	Pump flow	200-400 ml/min
	Maximum pressure	4 bar (60 psi)
	Maximum temperature	60°C

**Performance characteristics**

**Time to concentrate up to 20x [min.] at 3 bar inlet pressure, 20°C**

	<b>1 litre start volume</b>	<b>Average flux ml/min</b>	<b>Recovery % direct</b>	<b>25 ml rinse</b>
<b>Lysozyme 0.25 mg/ml (14,000 MW)</b>				
2,000 MWCO Hydrosart	160	6	97%	> 99%
3,000 MWCO PES	180	5	97%	> 99%
<b>BSA 1.0 mg/ml (66,000 MW)</b>				
5,000 MWCO PES	29	33	98%	> 99%
5,000 MWCO Hydrosart	70	14	98%	> 99%
10,000 MWCO PES	23	41	96%	> 99%
10,000 MWCO RC	42	23	97%	> 99%
10,000 MWCO Hydrosart	35	27	98%	> 99%
30,000 MWCO PES	25	38	96%	99%
30,000 MWCO Hydrosart	20	48	96%	> 99%
50,000 MWCO PES	22	43	96%	98%
<b>γ Globulins 1.0 mg/ml (average 160,000 MW)</b>				
100,000 MWCO PES	54	18	96%	99%
100,000 MWCO RC	45	21	96%	99%
<b>Yeast 1.0 mg/ml (S. Cerevisiae)</b>				
0.2 μm PES	11	86	92%	98%
<b>Dilute solute concentration, start volume 1 litre at 3 bar, 10,000 MWCO PES</b>				
BSA 0.001 mg/ml	18	52	90%	98%
BSA 0.01 mg/ml	20	47	92%	98%
BSA 0.1 mg/ml	21	45	94%	99%
<b>Start volume 5 litres (two VF200 in parallel at 3 bar) 10,000 MWCO PES</b>				
BSA 1.0 mg/ml (66,000 MW)	67	70	97%	> 99%

**Operation – Single Module****Operation – Two Modules****Ordering information**

**Vivaflow 200 modules include pressure indicator, flow restrictor and size 16 pvc peristaltic tubing and fittings**

	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
3,000 MWCO PES	1	VF20P9	–
5,000 MWCO PES	1	VF20P1	14-558-319
10,000 MWCO PES	1	VF20P0	14-558-318
30,000 MWCO PES	1	VF20P2	14-558-320
50,000 MWCO PES	1	VF20P3	14-558-321
100,000 MWCO PES	1	VF20P4	14-558-322
0.2 µm PES	1	VF20P7	14-558-323
10,000 MWCO RC	1	VF20C0	14-558-313
100,000 MWCO RC	1	VF20C4	14-558-314
5,000 MWCO Hydrosart	1	VF20H1	14-558-316
10,000 MWCO Hydrosart	1	VF20H0	14-558-315
30,000 MWCO Hydrosart	1	VF20H2	14-558-317

**Vivaflow 200 complete system comprises:**

Pump (115 V), Easy load pump head (size 16), tubing, 500 ml sample   diafiltration reservoir	1	VFS204	14-558-340
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**Vivaflow 200 accessories**

Masterflex economy drive variable speed peristaltic pump (115 V)	VFP002	14-558-339
500 ml sample and   or diafiltration reservoir	VFA006	14-558-327
Masterflex easy load pump head – size 16	VFA012	14-558-329
Masterflex easy load pump head – size 15	VFA013	14-558-330

**Vivaflow 200 tubing and fittings**

Size 15 pvc pump tubing and Luer fittings (3 m, 4.8 × 2.6 mm)	VFA003	14-558-324
Size 16 pvc pump tubing and Luer fittings (3 m, 3.2 × 1.6 mm)	VFA004	14-558-325
Y connector (size 15 to 2 × size 16)	VFA005	14-558-326
Flow restrictor set (2 × 0.4, 0.6, 0.8 mm)	VFA009	14-558-328
Female luer fittings size 16 (10 pieces)	VFA032	14-558-335
Flow restrictors 0.6 mm (6 pieces)	VFA035	14-558-337
Female luer fittings size 15 (10 pieces)	VFA036	14-558-338

## Vivapore Solvent Absorption Concentrators



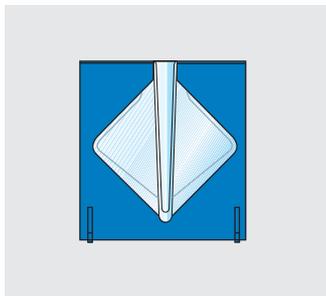
### 0.5 ml–20 ml samples

With no need for additional equipment, pressure or vacuum, solvent absorption is the most economic and user friendly concentration technique available to the clinician and research scientist.

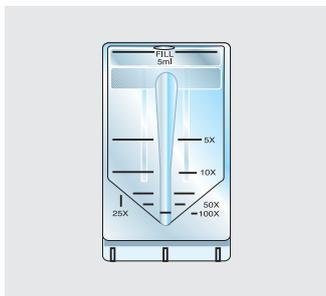
Just fill the unit with the solution to be concentrated, wait for the desired concentration level to be achieved and then pipette the concentrated sample from the bottom of the reservoir.

Vivapore is ideal for general purpose laboratory concentration or purification prior to further analysis. It is particularly suited for labile solutions that can denature with alternative shear or pressure inducing methods or that require processing in a cold room environment.

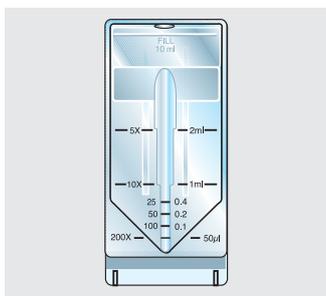
Vivapore concentrators extend the solvent absorption technique to a totally new level of performance, application potential and ease of use.



Vivapore 2



Vivapore 5



Vivapore 10|20

### Technical specifications

	Vivapore 2	Vivapore 5	Vivapore 10 20
Membrane material	PES	PES	PES
Membrane MWCO	7,500	7,500	7,500
Membrane surface area	15 cm <sup>2</sup>	20 cm <sup>2</sup>	28 cm <sup>2</sup>
Reservoir material	SAN	SAN	SAN
Volume range	0.5–2.5 ml   15 ml*	1–5 ml	2–10 ml   20 ml*
Minimum concentrate volume	20 µl	50 µl	50 µl
Vivapore overall dimensions			
Width (mm)	66	42	46
Height (mm)	68	82	100

### Performance characteristics

Product	Time to concentrate up to 10x [min.]				Concentrate recovery %			
	VP2	VP5	VP10 20	VP10 20*	VP2	VP5	VP10 20	VP10 20*
Start volume	2 ml	5 ml	10 ml	20 ml	2 ml	5 ml	10 ml	20 ml
Cytochrome c (12,600 MW)	0.25 mg/ml	0.25 mg/ml	0.25 mg/ml	0.1 mg/ml	0.25 mg/ml	0.25 mg/ml	0.25 mg/ml	0.1 mg/ml
7,500 MWCO PES	35	35	75	150	90%	90%	90%	92%
BSA (66,000 MW)								
7,500 MWCO PES	25	30	55	115	90%	92%	92%	92%
IgG (160,000 MW)								
7,500 MWCO PES	35	40	70	160	76%	75%	77%	78%
Product	Time to concentrate up to 50x [min.]				Concentrate recovery %			
	VP2	VP5	VP10 20	VP10 20*	VP2	VP5	VP10 20	VP10 20*
Cytochrome c (12,600 MW)								
7,500 MWCO PES	65	70	160	–	91%	88%	90%	–
BSA (66,000 MW)								
7,500 MWCO PES	45	50	105	218	90%	90%	92%	94%
IgG (160,000 MW)								
7,500 MWCO PES	50	65	140	290	53%	65%	74%	70%

\* with additional reservoir

**Ordering information**

<b>Vivapore 2</b> Expandable to 15 ml with pipette reservoir	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
7,500 MWCO PES	30	VP0201	14-558-384

**Vivapore 5**  
 Includes stand and recovery pipettes

7,500 MWCO PES	4	VP0503	14-558-387
7,500 MWCO PES	30	VP0501	14-558-385

**Requires stand**

7,500 MWCO PES	100	VP0502	14-558-386
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**Vivapore 10 | 20**  
 Includes stand and recovery pipettes

7,500 MWCO PES	4	VP2003	14-558-390
7,500 MWCO PES	30	VP2001	14-558-388

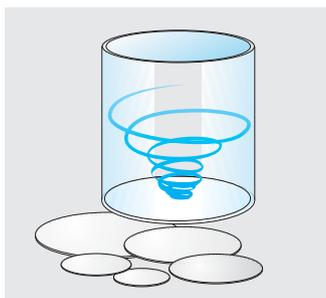
**Requires stand**

7,500 MWCO PES	100	VP2002	14-558-389
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**Vivapore accessories**

Disposable stands for 4 units	6	VPA002	14-558-391
Pipette reservoir (Vivapore 2)	50	VPA004	14-558-392
Plastic recovery pipettes (Vivapore 10   20)	100	VPA005	14-558-393
10 ml expansion reservoir (Vivapore 10   20)	10	VPA006	14-558-394
Plastic recovery pipettes (Vivapore 5)	100	VPA007	14-558-395

## Ultrafiltration Membrane Discs



Sartorius Stedim Biotech offers a broad range of ultrafiltration membrane discs for sample concentration in your stirred cells. You can choose from membrane discs from 13 mm to 300 mm in diameter, pore sizes from 1 to 300 kDa and three different membrane materials depending on your application.

### PES

low fouling characteristics  
pore sizes 1 kDa – 300 kDa

### CTA

high hydrophilicity  
high recovery for filtrate  
pore sizes 5, 10 and 20 kDa

### Hydrosart

extremely low protein binding  
extended chemical resistance  
pore sizes 2, 5, 10 and 30 kDa

### Performance characteristics for Polyethersulfone, type 146

Thickness	120 µm	
pH range	1–14	
Waterflux*	MWCO 10,000	0.2 ml/min/cm <sup>2</sup>
Protein retention	Cytochrome c	95%

### Performance characteristics for Cellulose Triacetate, type 145

Thickness	120 µm	
pH range	4–8	
Waterflux*	MWCO 10,000	0.11 ml/min/cm <sup>2</sup>
Protein retention	Cytochrome c	90%

### Performance characteristics for Hydrosart®, type 144

Thickness	180 µm	
pH range	1–13	
Waterflux*	MWCO 10,000	0.08 ml/min/cm <sup>2</sup>
Protein retention	Cytochrome c	99%

\* Measured at 4°C and 4 bar

## Ordering Information

Sartorius Stedim Order No.	Fisher Scientific Order No.	Diameter (mm)	Membrane	MWCO	Pack size
14429--25-----D	14-558-225	25	Hydrosart®	5,000	10
14439--25-----D	14-558-230	25	Hydrosart®	10,000	10
14429--47-----D	14-558-227	47	Hydrosart®	5,000	10
14439--47-----D	14-558-231	47	Hydrosart®	10,000	10
14459--47-----D	14-558-235	47	Hydrosart®	30,000	10
14429--63-----D	14-558-228	63	Hydrosart®	5,000	10
14439--63-----D	14-558-232	63	Hydrosart®	10,000	10
14459--63-----D	14-558-236	63	Hydrosart®	30,000	10
14429--76-----D	14-558-229	76	Hydrosart®	5,000	10
14439--76-----D	14-558-233	76	Hydrosart®	10,000	10
14529--47-----D	■■■■	47	CTA	5,000	10
14539--47-----D	■■■■	47	CTA	10,000	10
14539--50-----D	■■■■	50	CTA	10,000	10
14549--43-----D	■■■■	43	CTA	20,000	10
14549--47-----D	■■■■	47	CTA	20,000	10
14549--47-----N	■■■■	47	CTA	20,000	100
14629--25-----D	14-558-242	25	PES	5,000	10
14639--25-----D	14-558-247	25	PES	10,000	10
14609--47-----D	14-558-239	47	PES	1,000	10
14629--47-----D	14-558-243	47	PES	5,000	10
14639--47-----D	14-558-249	47	PES	10,000	10
14650--47-----D	14-558-253	47	PES	50,000	10
14659--47-----D	14-558-257	47	PES	30,000	10
14668--47-----D	14-558-260	47	PES	100,000	10
14679--47-----D	14-558-262	47	PES	300,000	10
14629--63-----D	14-558-244	63	PES	5,000	10
14639--63-----D	14-558-250	63	PES	10,000	10
14659--63-----D	14-558-258	63	PES	30,000	10
14668--63-----D	■■■■	63	PES	100,000	10
14629--76-----D	14-558-245	76	PES	5,000	10
14639--76-----D	14-558-251	76	PES	10,000	10

## Vivacon® 500

### For DNA sample desalting and concentration



#### Reproducible DNA and protein sample desalting and concentration

Vivacon® 500 centrifugal concentrators offer the optimal solution for DNA and protein concentration and buffer exchange applications. For optimal performance with very dilute samples, e.g. forensic samples, Vivacon® 500 is equipped with the patented regenerated cellulose membrane Hydrosart®.

High recoveries and excellent reproducibilities are paired with convenience offered by molecular weight cut-off printed on individual devices.

The possibility of a re-spin after sample processing assures complete concentrate recovery which is especially important when working with low sample concentrations.

#### New: Vivacon® 500-PCR Grade

When using DNA amplification technologies, any traces of DNA originating from the equipment have to be eliminated.

Vivacon® 500-PCR Grade units are treated with ethylene oxide (ETO) in a validated process in order to deactivate all traces of DNA that might interfere with subsequent amplification procedures.

Ref.: K. Shaw et al., Int. J. Legal Med. (2008) 122: 29-33

Feature	Benefit
Re-spin possibility	Complete and highly reproducible sample recovery
Low binding material	High recoveries of low sample concentrations

#### Technical Specifications Vivacon® 500

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 <sup>2</sup>
	Hold up volume of membrane and support	< 5 µl
	Dead stop volume (40° rotor)	5 µl
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polypropylene
	Membrane	Hydrosart®

**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

**Performance Characteristics 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

**Performance Characteristics 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

## Ordering Information

Vivacon® 500	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
2,000 MWCO	25	VN01H91	14-558-358
2,000 MWCO	100	VN01H92	14-558-359
10,000 MWCO	25	VN01H01	14-558-343
10,000 MWCO	100	VN01H02	14-558-344
30,000 MWCO	25	VN01H21	14-558-345
30,000 MWCO	100	VN01H22	14-558-347
50,000 MWCO	25	VN01H31	14-558-350
50,000 MWCO	100	VN01H32	14-558-351
100,000 MWCO	25	VN01H41	14-558-353
100,000 MWCO	100	VN01H42	14-558-355

Vivacon® 500	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
Sample Kit L (4 units each of 2, 10, 30 K)	12	VN01HL12	■■■■
Sample Kit H (4 units each of 30, 50, 100 K)	12	VN01HH12	■■■■

Vivacon® 500-PCR Grade	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
30,000 MWCO	25	VN01H21ETO	14-558-346
30,000 MWCO	100	VN01H22ETO	14-558-348
100,000 MWCO	25	VN01H41ETO	14-558-354
100,000 MWCO	100	VN01H42ETO	14-558-356

## Vivacon® 2

### For DNA sample desalting and concentration



#### Reproducible DNA sample desalting and concentration

Vivacon® 2 centrifugal concentrators offer the optimal solution for DNA and protein concentration and buffer exchange applications. For optimal performance with very dilute samples, e.g. forensic samples, Vivacon® 2 is equipped with the patented regenerated cellulose membrane Hydrosart®.

#### New: Vivacon® 2-PCR Grade

Vivacon® 2-PCR Grade units are treated with ethylene oxide (ETO) in a validated process in order to deactivate all traces of DNA that might interfere with subsequent amplification procedures.

High recoveries and excellent reproducibilities are paired with convenience offered by volume graduation and molecular weight cut-off printed on individual devices.

The possibility of a re-spin after sample processing assures complete concentrate recovery which is especially important when working with low sample concentrations.

Feature	Benefit
Re-spin possibility	Complete and highly reproducible sample recovery
Low binding material	High recoveries of low sample concentration
Easy to remove re-spin cap	Convenient sample handling
Graduation printed on	Optimal process control

#### Technical Specifications

Concentrator capacity	Fixed angle rotor	2 ml
Dimensions	Total length (Concentration)	125 mm
	Total length (Back-spin)	115 mm
	Width	16 mm
	Active membrane area	0.95 cm <sup>2</sup>
	Hold-up volume membrane and support	10 µl
	Dead stop volume (25° rotor)	55 µl
Materials of construction	Body	Polycarbonate
	Filtrate vessel	Polypropylene
	Back spin vial	Polypropylene
	Concentrator cap	Polypropylene
	Membrane	Hydrosart®

**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

**Performance Characteristics**

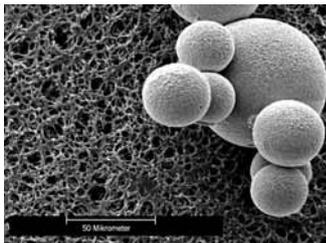
Volume 2 ml, sample concentration 50 ng/ml, Start volume: 2 ml

	Sample size (bp)	Time to concentrate up to 30x [min.] at 20°C	Concentrate recovery %	g-force (xg)
2,000 MWCO	10	120 min	92%	7,500
10,000 MWCO	30	60 min	94%	5,000
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

**Ordering Information**

Vivacon® 2	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
2,000 MWCO	25	VN02H91	14-558-379
2,000 MWCO	100	VN02H92	14-558-380
2,000 MWCO	500	VN02H93	14-558-381
10,000 MWCO	25	VN02H01	14-558-360
10,000 MWCO	100	VN02H02	14-558-361
10,000 MWCO	500	VN02H03	14-558-362
30,000 MWCO	25	VN02H21	14-558-364
30,000 MWCO	100	VN02H22	14-558-365
30,000 MWCO	500	VN02H23	14-558-367
50,000 MWCO	25	VN02H31	14-558-369
50,000 MWCO	100	VN02H32	14-558-370
50,000 MWCO	500	VN02H33	14-558-371
100,000 MWCO	25	VN02H41	14-558-373
100,000 MWCO	100	VN02H42	14-558-374
100,000 MWCO	500	VN02H43	14-558-376
Vivacon® 2-PCR Grade	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
30,000 MWCO	25	VN02H21ETO	■■■■
30,000 MWCO	100	VN02H22ETO	14-558-366
100,000 MWCO	25	VN02H41ETO	■■■■
100,000 MWCO	100	VN02H42ETO	14-558-375

## Vivapure® Ion Exchange Protein Purification Products



Chromatography gel beads (right) are shown on top of a membrane adsorber in this SEM picture. The membrane adsorber pores are over 50 × larger than bead pores.

### Fast and easy-to-use spin columns

Vivapure Ion Exchange (IEX) spin columns are centrifugal devices, incorporating Sartobind Membrane Adsorber technology as their chromatography matrix. Vivapure IEX spin columns make protein purification as easy as filtration. The devices are ready-to-use and do not bear the risk of running dry. For many protein purification applications, they can replace time-consuming and tedious column chromatography.

The rapid 1-2-3 bind-wash-elute protocol especially lends itself to screening applications, where many different samples are processed in parallel.

### The Sartobind membrane adsorber matrix

Sartobind IEX membrane adsorbers are based on stabilized regenerated cellulose and display a microporous structure with a pore size of > 3 μm, which is orders of magnitude larger than conventional chromatographic gel materials. This allows molecules to be transported to the ligands immobilized on the membrane adsorber by convective flow, leading to very high flow rates.

In contrast to that, gel chromatography is slowed down due to diffusion limitations, as the molecules need to enter the small bead pores in order to be bound by the ligands. The porous membrane adsorber enables fast, reproducible and scalable protein purification.

### Fast and simple to use spin columns

- Devices are ready to use
- Make protein purification as simple as filtration

### Reproducible results

- No column packing necessary – devices are ready to use
- Membrane adsorber spin columns cannot crack or run dry

### Centrifugal devices

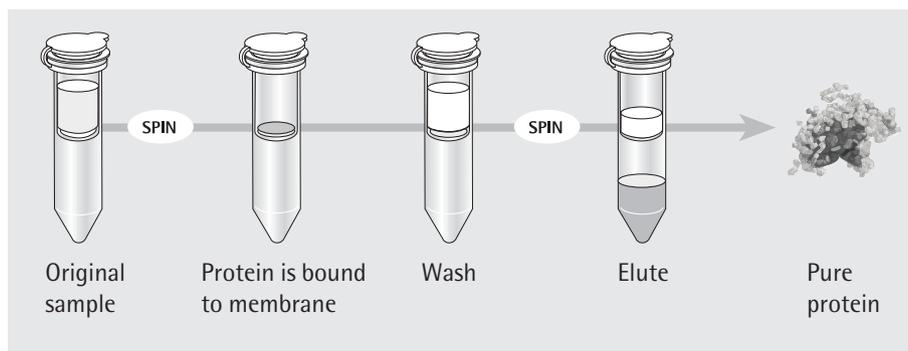
- Offer the possibility of working in parallel

### Low bed volume

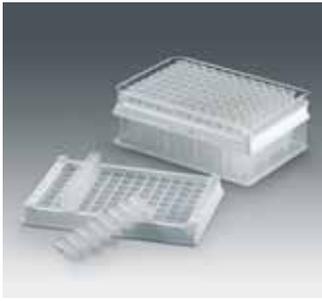
- Small membrane adsorber bed volumes allow working with lower buffer amounts, leading in concentrated elution fractions

### Up-scalable product range

- Process scale modules are available with the same Sartobind IEX membrane adsorber matrix



Fast and easy protein purification with Vivapure spin columns



Vivawell 8-strip plate-300 µl  
Binding capacity: 1 mg



Vivapure Mini-400 | 500 µl  
Binding capacity: 1–4 mg



Vivapure Maxi-19 | 20 ml  
Binding capacity: 15–80 mg

### Available formats

Vivapure® IEX Products	Application
Vivawell 8-strip plates	– High throughput application, where larger capacities are needed (e.g. high throughput applications for Vivapure Mini)
Vivapure Mini Spin Columns	– Sample fractionation – Purification condition scouting – Small scale purification
Vivapure Maxi Spin Columns	– Large scale sample fractionation – One step protein purification   concentration – Polishing of his-tagged protein

### Membrane availability

Functional groups	Ion exchanger type
Sulphonic acid (S)	Strong acidic cation exchanger: $R-CH_2-SO_3^-Na^+$
Quaternary ammonium (Q)	Strong basic anion exchanger: $R-CH_2-N^+-(CH_3)_3Cl^-$
Diethylamine (D)	Weak basic anion exchanger: $R-CH_2-NH^+-(CH_2H_5)_2$

### Performance characteristics

Vivapure spin columns	Protein binding capacity* (mg)	Max. volume per centrifuge run using a swing-out rotor (ml)	Max. volume per centrifuge using a fixed angle rotor run (ml)
Vivawell 8-strip	1	0.3	
Vivapure Mini M	1	0.5	
Vivapure Mini H	4	0.4	
Vivapure Maxi M	15–20	20	10.5
Vivapure Maxi H	60–80	19	10.5

\* Actual yields depend on specific protein sample and selected pH and salt conditions. Yields established using 1 mg/ml BSA in 25 mM Tris/HCL pH 8.0 with Vivapure Q & D spin columns and 1 mg/ml cytochrome c in 25 mM sodium acetate buffer pH 5.5 with Vivapure S spin columns.

### Typical Applications

- Fractionation prior to further analysis e.g. 2D gels
- Scouting purification conditions for new protein preparation protocols
- Endotoxin removal
- Polishing His-tagged proteins after metal chelate chromatography
- Purification and concentration of proteins
- Removal of heme moiety from heme containing proteins

Detailed application notes are available on our website: [www.sartorius-stedim.com](http://www.sartorius-stedim.com)

### Ordering Information

<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>	<b>Description</b>	<b>Spin Columns</b>	<b>Centrifuge Tubes</b>
<b>Vivapure Mini Ion Exchange Spin Columns (up to 0.5 ml)</b>				
VS-IX01SQ16	14-558-557	Vivapure Mini S&Q H starter kit	16	32
VS-IX01DM24	14-558-552	Vivapure D Mini M	24	48
VS-IX01DH24	14-558-551	Vivapure D Mini H	24	48
VS-IX01QM24	14-558-554	Vivapure Q Mini M	24	48
VS-IX01QH24	14-558-553	Vivapure Q Mini H	24	48
VS-IX01SM24	14-558-556	Vivapure S Mini M	24	48
VS-IX01SH24	14-558-555	Vivapure S Mini H	24	48
<b>Vivapure Maxi Ion Exchange Spin Columns (up to 20 ml)</b>				
VS-IX20DH08	14-558-558	Vivapure D Maxi H	8	16
VS-IX20QM08	14-558-560	Vivapure Q Maxi M	8	16
VS-IX20QH08	14-558-559	Vivapure Q Maxi H	8	16
VS-IX20SM08	14-558-562	Vivapure S Maxi M	8	16
VS-IX20SH08	14-558-561	Vivapure S Maxi H	8	16
<b>Vivawell 8-Strip</b>				<b>Pack Size</b>
VW08ID02	14-558-577	Vivawell 8-Strip D	24	
VW08IS02	14-558-580	Vivawell 8-Strip S	24	
VW08IQ02	14-558-578	Vivawell 8-Strip Q	24	

## Vivawell Vac Vacuum Manifold systems



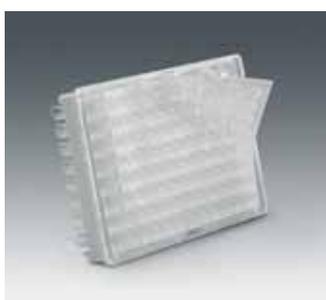
Vivawell Vac96 set-up



Vivawell Vac 96



Vivawell Vac8



Vivawell Vac8-strip plate

### New Vivawell Vac8 and Vivawell Vac96 Vacuum Manifold systems

The new Vivawell-Vac vacuum manifolds have been designed specifically for use with Vivawell Vac 8-strip units and plates.

The extra long drip nozzles on the 8-strip outlet eliminate gaps between the sample flow and receiver wells. This direct stacking prevents cross talk between individual wells. Vivawell Vac96 can be easily configured for both flow-to-waste and analyte collection.

The system is easy to use with quick release fitting and can be run without initial set up time.

The Vivawell Vac8 and 96 vacuum manifold features:

- Cross-talk free filtration due to extra long drip nozzels
- Configurations for 1 ml and 2 ml collection plates with adaptor

### Specifications for Vivawell Vac96

Manifold assembly	1
Quick release vacuum fitting	1
Control valve	1
Vacuum Tubing	1 m
Hose barb fitting	1

### Materials of construction

Manifold Base + Adaptor ring	Acetal
Manifold Top Plate	Anodised Aluminium
O-ring	Silicone
Quick release vacuum fitting	Acetal
Manifold dimensions (W×D×H)	144×102×71

### Ordering information

Sartorius Stedim Order No.	Fisher Scientific Order No.	Description	Pack size
VW96VAC01	14-558-592	Vivawell Vac96	1
VW96VAA02	14-558-588	Vivawell Vac96 liquid trap and reservoir	1
VW96VAA03	14-558-589	96 deep well collection plate 1 ml (square wells)	25
VW96VAA04	14-558-590	96 deep well collection plate 2 ml (square wells)	25
VW96VAA05	14-558-591	Replacement seal for Vivawell Vac96	1

### Required Equipment

- Vivawell Vac96
- Vivawell Vac 8-strip plate
  - Vacuum pump or vacuum source capable of applying vacuum at 10" Hg or higher

**Specifications for Vivawell Vac8**

Manifold assembly	1
Quick release vacuum fitting	1
Control valve	1
Vacuum Tubing	1 m
Hose barb fitting	1
8-well collection strips (1.2 ml)	5
Single strip silicone gaskets	12

**Materials of construction**

Manifold Base + Adaptor ring	Acetal
Manifold Top Plate	Clear acrylic
O-ring	Silicone
Quick release vacuum fitting	Acetal
Manifold dimensions (W×D×H)	105×80×58

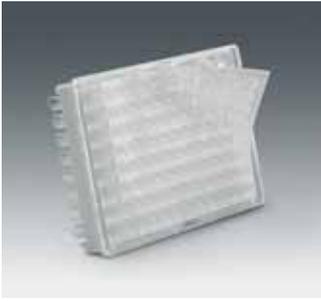
**Ordering information**

<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>	<b>Description</b>	<b>Pack size</b>
VW08VAC01	14-558-585	Vivawell Vac8	1
VW08VAA02	14-558-582	Vivawell Vac8 liquid trap and reservoir	1
VW08VAA03	14-558-583	8 well collection strips 1.2 ml (round wells)	125
VW08VAA04	14-558-584	Replacement seal for Vivawell Vac8	1

**Required Equipment**

Vivawell Vac8	<ul style="list-style-type: none"> <li>- Vivawell Vac 8-strip units</li> <li>- Vacuum pump or vacuum source capable of applying vacuum at 10" Hg or higher</li> </ul>
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## Vivawell Vac 8-strip plate



Vivawell Vac8-strip plate

- For use with Vivawell Vac Vacuum manifold systems
- Optimal for high-throughput applications
- Flexibility in number of samples to be processed

Vivawell Vac 8-strips feature a modular design of individual 8-strip units set into a 96-well frame. A silicone gasket seals the plate set-up of 12 individual 8-strip units, for vacuum processing.

Using Vivawell Vac8, individual 8-strips can be run for medium throughput applications.

The Vivawell Vac 8-strip IEX plate is available with two different membrane functionalities and can be processed as a 96-well plate with the Vivawell Vac96 (Sartorius Stedim Order No. VW96VAC01 | Fisher Scientific Order No. 14-558-592) or as individual 8-strips with the Vivawell Vac8 (Sartorius Stedim Order No. VW08VAC01 | Fisher Scientific Order No. 14-558-585).



Vivawell Vac8-strip plate on Vivawell Vac 96

For large sample quantities, the full plate set-up can be processed quickly with Vivawell Vac96.

### Membrane availability

Functional groups	Ion exchange type	
Sulphonic acid (S)	Strong acidic cation exchanger	$R-CH_2-SO_3^-Na^+$
Quaternary ammonium (Q)	Strong basic anion exchanger	$R-CH_2-SO_3^-Na^+-(CH_2)_2Cl^-$

### Membrane Adsorber

Nominal pore size	3 – 5 $\mu m$ (Large pore size prevents gel filtration effects and minimizes non-specific adsorption)
Thickness	230 – 320 $\mu m$
Amount of ionic groups ( $\mu$ -Equivalents/ml)	145 – 218 $\mu$ -Equivalents/ml for monovalent ions (Q&S)
Working pH (Q&S)	2 – 12
Approximate pKa of ionic groups	Q-11   S-1



Vivawell Vac8-strip on Vivawell Vac8

**Materials of construction**

Vivawell 8-strip IEX units	Polypropylene
Supporting matrix	Stabilized regenerated cellulose
Holding Frame	Polypropylene

**Capacities and dimensions**

Device	Bed Volume ( $\mu$ l)	Membrane Area ( $\text{cm}^2$ )
Vivawell Vac 8-strip	80	2.4

**Ordering information**

Sartorius Stedim Order No.	Fisher Scientific Order No.	Description	Pack size
VW08IQ02V	14-558-579	Vivawell Vac 8-strip Q-IEX purification strips	24
VW08IS02V	14-558-581	Vivawell Vac 8-strip S-IEX purification strips	24

**Required Equipment**

Vivawell Vac 8-strip IEX plate	<ul style="list-style-type: none"> <li>- Vivawell Vac manifold (Sartorius Stedim Order No. VW96VAC01/VW08VAC01   Fisher Scientific Order No. 14-558-592/14-558-585)</li> <li>- Vacuum pump or vacuum source capable of applying vacuum at 10" Hg or higher</li> <li>- Vivawell Vac system liquid trap</li> </ul>
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## Vivapure® mini & maxiprep Purification Kits for a fast Antibody and His-Tagged Protein Purification



**Rapid Purification with High Yields**  
Vivapure® miniprep and maxiprep kits are spin column based kits for fast and effective purification of His-tagged proteins and antibodies.

Spin columns have the advantage of speed over gravity drip columns and batch protocols.

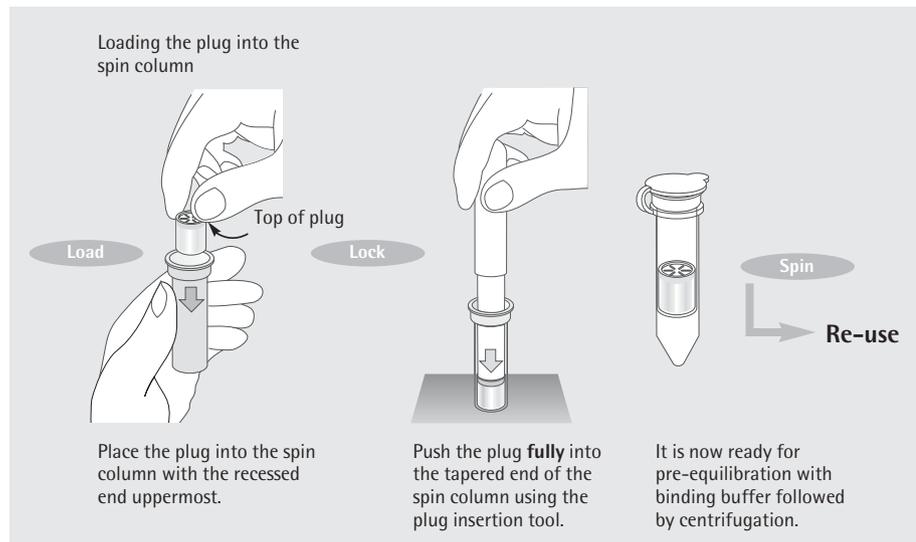


With the patented FlowGo regulator the sample residence time is extended to assure adequate sample binding to the resin. Due to this, Vivapure® miniprep and maxiprep spin column kits combine the merits of spin columns and gravity drip columns resulting in rapid purification with up to 95% protein recovery and purity.

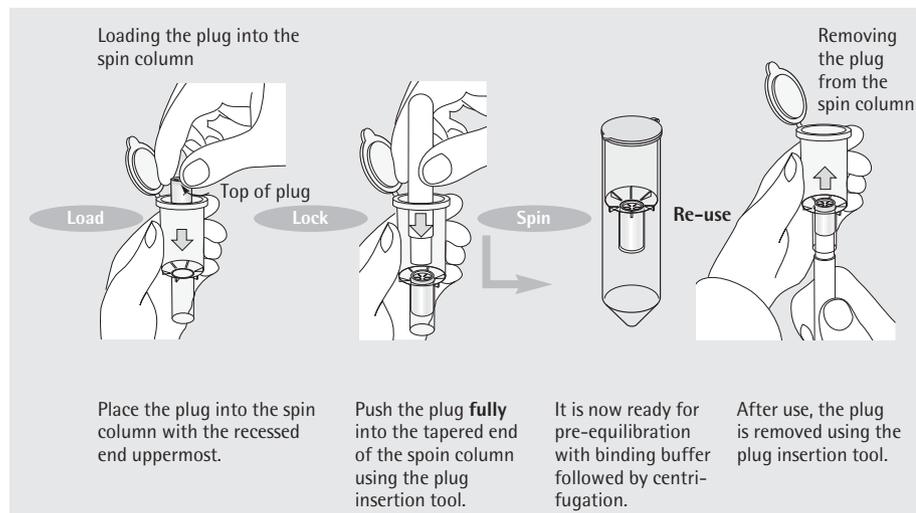
All spin columns can conveniently be used in a centrifuge. For processing larger sample volumes, e. g. from diluted cell culture supernatants, the Vivapure® maxiprep spin columns can additionally be run with a peristaltic pump collar ([Sartorius Stedim Order No. VS-PPCSC](#) | [Fisher Scientific Order No. 14-558-572](#)).

The Vivapure® miniprep and maxiprep columns come in a variety of different configurations for your convenience. They can be purchased as completely ready-to-use kits with buffers and ultrafiltration devices but just as well as stand alone spin columns in small packs or large packs for frequent users.

### Vivapure® miniprep



### Vivapure® maxiprep



## Vivapure® mini|maxiprep Protein A & G Spin Columns



Affinity purification of monoclonal antibodies has been largely confined to the use of Protein A and Protein G chromatography. The Vivapure® mini|maxiprep A & G kits are designed for simple, rapid antibody purification from serum, ascites and tissue culture supernatant such as those derived from static cultures and bioreactors. Vivapure® mini|maxiprep spin columns replace lengthy and expensive chromatographic methods such as FPLC.

Large numbers of samples can be processed in parallel. The low hold-up volume ensures high solute recovery with minimal non-specific absorptive losses.

### The Vivapure® mini|maxiprep A & G Advantages

- Spin column based kits for fast results
- Parallel processing
- Different configurations to suit all needs
- Economical due to re-usability

### Working with samples > 20 ml Accessory

For working with sample volumes larger than 20 ml, e.g. diluted cell culture supernatants, a sealing cap and peristaltic pump collar (Sartorius Stedim Order No. VS-PPCSC | Fisher Scientific Order No. 14-558-572) for Vivapure® maxiprep columns offer a fast and easy to use alternative to multiple centrifugation steps. The sample is pumped into the maxiprep spin column through a tube attached to the sealing cap with a connector. To ensure the usage of high sample loading flow rates, the peristaltic pump collar securely locks the sealing cap to the column barrel.

### Required Equipment

Variable speed peristaltic pump capable of speeds less than 20 rpm. E. g. Masterflex pump (Sartorius Stedim Order No. VFP002 | Fisher Scientific Order No. 14-558-339, 115 V), Masterflex easy load pump head-size 16, (Sartorius Stedim Order No. VFA012 | Fisher Scientific Order No. 14-558-329).

### Ordering information

Product name	Pack size	Sartorius Stedim Order No.	Fisher Scientific Order No.
Vivapure A Starter Pack*	2 miniprepA	VS-ARSTPKA2	14-558-545
Vivapure miniprepA Kit*	16 miniprepA	VS-ARAMINIK	14-558-539
Vivapure miniprepA Bulk Pack	48 miniprepA	VS-ARAMINIB	14-558-538
Vivapure maxiprepA Kit*	4 maxiprepA	VS-ARAMAXIK	14-558-537
Vivapure maxiprepA Bulk Pack	12 maxiprepA	VS-ARAMAXIB	14-558-536
Vivapure A Buffer Pack		VS-ARABUFPK	14-558-535
Vivapure G Starter Pack*	2 miniprepG	VS-ARSTPKG2	14-558-546
Vivapure miniprepG Kit*	16 miniprepG	VS-ARGMINIK	14-558-544
Vivapure miniprepG Bulk Pack	48 miniprepG	VS-ARGMINIB	14-558-543
Vivapure maxiprepG Kit*	4 maxiprepG	VS-ARGMAXIK	14-558-542
Vivapure maxiprepG Bulk Pack	12 maxiprepG	VS-ARGMAXIB	14-558-541
Vivapure G Buffer Pack		VS-ARGBUFPK	14-558-540
Sealing Cap & Peristaltic Pump Collor 1		VS-PPCSC	14-558-572

\* including UF-concentrators and buffers

## Technical Data

### Protein A & G for Antibody Purification

Protein A & G miniprep	Centrifuge
Sample size	0.65 ml
Typical Binding Capacity	1 mg IgG/column
Number of re-uses	3

Protein A & G maxiprep	Centrifuge <sup>1</sup>
Sample size	20 ml
Typical Binding Capacity	20 mg IgG/column
Number of re-uses	5

<sup>1</sup> Use the peristaltic pump accessory (Sartorius Stedim Order No. VS-PPCSC | Fisher Scientific Order No. 14-558-572) for larger volumes

### Binding Affinities of Protein A and Protein G

Antibody	Protein A	Protein G	Antibody	Protein A	Protein G
Human IgG1	****	****	Rat IgG2c	*	**
Human IgG2	****	****	Rabbit IgG	****	***
Human IgG3	×	****	Hamster IgG	*	**
Human IgG4	****	****	Guinea Pig IgG****		**
Human IgGA	**	×	Bovine IgG	**	****
Human IgGD	**	×	Sheep IgG	*   ×	**
Human IgGE	**	×	Goat IgG	*   ×	**
Human IgGM	**	×	Pig IgG	***	***
Mouse IgG1	*	**	Chicken IgG	×	*
Mouse IgG2a	****	****			
Mouse IgG2b	***	***			
Mouse IgG3	**	***			
Rat IgG2a	×	****			
Rat IgG2b	×	**			

\*\*\*\* = Strong Affinity  
 \*\*\* = Moderate Affinity  
 \*\* = Weak Affinity  
 \* = Slight Affinity  
 × = No Affinity

## Vivapure® mini|maxiprep MC Spin Columns



The Vivapure® mini|maxiprep MC kit is designed for simple, rapid His-tagged recombinant protein purification from a cell lysate under native or denaturing conditions. Vivapure® spin columns replace lengthy and expensive chromatographic methods such as FPLC®. Metal chelate affinity chromatography is a rapid onestep purification, which removes most contaminants and can achieve purities close to homogeneity.

This Vivapure® MC purification kit incorporates pre-packed Ni<sup>2+</sup>-IDA agarose resin plugs in ready-to-use spin columns. The objective is to offer the researcher total protein purification solutions from the initial fractionation stage to the final polishing steps. Resolution of the His-tagged protein is achieved either in a 2.2 ml microfuge tube for the Vivapure® Mini spin column or in a 50 ml centrifuge tube for the Vivapure® Maxi spin column.

### The Vivapure® mini|maxiprep MC Advantages

- Spin column based kits for fast results
- Parallel processing
- Different configurations to suit all needs
- Economical due to re-usability

### Working with samples > 20 ml Accessory

For working with sample volumes larger than 20 ml, e.g. diluted cell culture supernatants, a sealing cap and peristaltic pump collar (Sartorius Stedim Order No. VS-PPCSC | Fisher Scientific Order No. 14-558-572) for Vivapure® maxiprep columns offer a fast and easy to use alternative to multiple centrifugation steps. The sample is pumped into the maxiprep spin column through a tube attached to the sealing cap with a connector. To ensure the usage of high sample loading flow rates, the peristaltic pump collar securely locks the sealing cap to the column barrel.

### Required Equipment

Variable speed peristaltic pump capable of speeds less than 20 rpm. E. g. Masterflex pump (Sartorius Stedim Order No. VFP002 | Fisher Scientific Order No. 14-558-339, 115 V), Masterflex easy load pump head-size 16, (Sartorius Stedim Order No. VFA012 | Fisher Scientific Order No. 14-558-329).

### Applications

- Ready-to-use, robust and reproducible kits for purifying His-tagged proteins from bacteria, insect, mammalian and yeast cells under native or denaturing conditions in Molecular Biology, Biochemistry or Structural Biology laboratories.
- Easy screening for soluble expression of His-tagged proteins
  - Run Vivapure® maxiprep MC columns in centrifugal mode for volumes < 20 ml or with peristaltic pump for > 20 ml
  - Purification of recombinant proteins for use as substrates for enzyme assays, structural studies of for raising antibodies
  - Titration of His-tagged protein yield during cell culture
  - Remove free His-tag after cleavage from purified recombinant protein
  - Multiple expression profiling to generate critical cell culture performance data and for predicting optimal harvest times

## Technical Data

<b>Protein MC miniprep Kits</b>	<b>Centrifuge</b>
Sample size	0.65 ml
Typical Binding Capacity	1 mg His-tagged protein
Number of re-uses	2

<b>Protein MC maxiprep Kits</b>	<b>Centrifuge<sup>1</sup></b>
Sample size	20 ml
Typical Binding Capacity	10 mg His-tagged protein
Number of re-uses	2

<sup>1</sup> Use the peristaltic pump accessory ([Sartorius Stedim Order No. VS-PPCSC](#) | [Fisher Scientific Order No. 14-558-572](#)) for larger volumes

## Ordering information

<b>Product name</b>	<b>Pack size</b>	<b>Sartorius Stedim Order No.</b>	<b>Fisher Scientific Order No.</b>
Vivapure metal cheleate Starter Pack*	4	VS-MCST04	14-558-571
Vivapure miniprepMC Kit*	24	VS-MCMINI24	14-558-569
Vivapure miniprepMC Bulk Pack	72	VS-MCMINIB	14-558-570
Vivapure maxiprepMC Kit*	8	VS-MCMAXIK	14-558-568
Vivapure maxiprepMC Bulk Pack	24	VS-MCMAXIB	14-558-567
Vivapure metal chelate Buffer Pack		VS-MCBUFPK	14-558-566

\* including UF-concentrators and buffers

## Vivapure Anti-HSA/IgG Kits – for Human Albumin and Human Albumin/IgG Depletion



The Vivapure Anti-HSA and Anti-HSA/IgG kits are intended for biologists involved in the discovery of serum biomarkers that need highly specific albumin or albumin and IgG removal at single use pricing.

The Vivapure Albumin Depletion Kit is based on a unique antibody fragment for specific albumin removal.

The Albumin/IgG Depletion Kit uses a combination of the Anti-HSA antibody fragment and Protein G resin for depleting albumin and IgG.

Additionally, all buffers and spin tubes required for albumin and albumin/IgG removal from 12 x 20 µl samples of human serum are included as well as a recommended protocol for recovery of albumin or albumin and IgG and associated proteins.

### The Vivapure Advantage

- Highly specific antibody fragment based albumin removal
- Protein G based IgG removal
- Priced for single use – no risk of contamination



Before



After\*

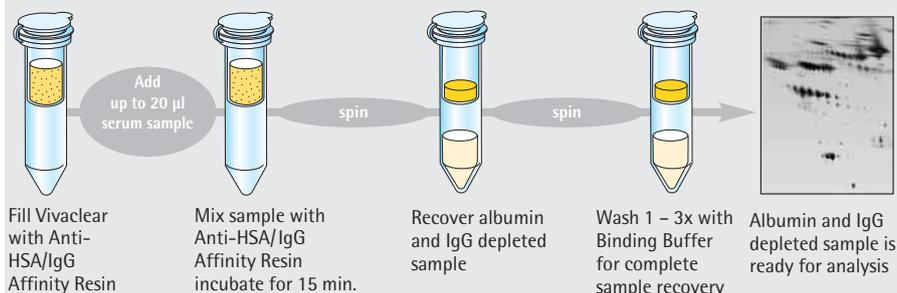
### Ordering Information

Sartorius Stedim Order No.	Scientific Fisher Order No.	Kit Contents
VS-SP08HAR	14-558-575	Vivapure Anti-HSA Kit for Human Albumin Depletion Anti-HSA Affinity Resin (50% slurry) 5 ml Clarification spin columns (Vivaclear) 12 Collection tubes (2 ml) 24 Binding Buffer 15 ml
VS-SP50HAR	14-558-576	Vivapure Anti-HSA Affinity Resin for Human Albumin Depletion Anti-HSA Affinity Resin (50% slurry) 50 ml
VS-SP08HAIGG	14-558-574	Vivapure Anti-HSA/IgG Kit for Human Albumin and IgG Depletion Anti-HSA/IgG Affinity Resin (50% slurry) 5.5 ml Clarification spin columns 12 Collection tubes (2 ml) 24 Binding Buffer 15 ml

### Specifications: Vivapure Anti-HSA and Anti-HSA/IgG Kits

Anti-HSA Affinity Resin binding capacity (suspension containing 50% packed medium)	2 mg/ml
Anti-HSA/IgG Affinity Resin binding capacity (suspension containing 50% packed medium)	1.8 mg/ml albumin 0.6 mg/ml IgG
Clarification spin columns (Vivaclear) max. volume capacity	500 µl
Recommended centrifugation speed	400 x g

### Handling overview – Albumin and Albumin/IgG removal in 20 minutes



## Vivapure C18 Micro Spin Columns



### Fast sample preparation for mass spectrometry

Vivapure C18 Micro spin columns are centrifugal membrane-based devices for concentration, purification and desalting of peptides prior to analysis by mass spectrometry. The columns are prepacked with a membrane containing C18 hydrophobic chains for reversed-phase chromatography.



The columns are the size of standard microcentrifuge tubes. With Vivapure C18 micro spin columns, a few centrifugation steps replace the tedious repetitive pipetting procedure for sample preparation prior to MALDI MS analysis. Samples are easily processed in parallel, and the tiny elution volumes are thoroughly collected in the included microtubes.

The Vivapure C18 spin columns offer a very fast and effective method to simultaneously desalt and concentrate up to 200  $\mu$ l of highly dilute peptide solutions from any source (2D-PAGE, chromatographic methods or biological samples).

### The Vivapure Advantage

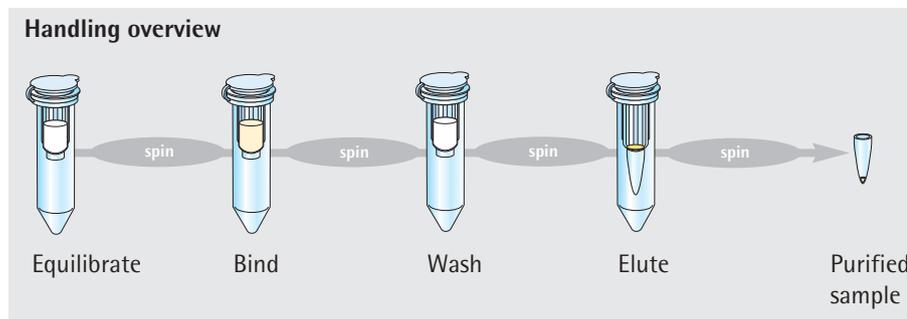
- Centrifugal format
- High volume capacity
- Low elution volume
- Parallel processing
- High reproducibility
- Elution in Matrix

### Ordering Information

Sartorius Stedim Order No.	Scientific Fisher Order No.	Kit Contents
VS-RP218L24	14-558-573	Vivapure C18 Micro spin columns
		Vivapure C18 Micro spin columns 24
		Micro collection tubes (200 $\mu$ l) 24
		Collection tubes (2 ml) 48

### Specifications

Binding capacity (for standard digestion)	5 $\mu$ g
Maximum volume	200 $\mu$ l
Minimum elution volume	3 $\mu$ l



## Vivapure® Virus Purification and Concentration Kits

Recombinant virus vectors are the preferred method for a wide range of gene delivery applications. Especially **adenovirus type 5** and **VSV-G pseudotyped lentivirus** are two frequently utilized viral vectors for in vitro and in vivo applications.

**Recombinant adenovirus vectors** are versatile tools in research and therapeutic applications for gene transfer and protein expression in cell lines that have low transfection efficiency with liposomes. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome, leaving the host genome unaffected). The delivery of RNAi into cells is becoming a major application for adenovirus vectors.

**Lentivirus vectors** are frequently used in gene transfer studies, due to their ability of gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens their target cell range. Lentiviral vectors have been shown to deliver genes into cell types (e.g. neurons, lymphocytes and macrophages) which other retrovirus vectors could not be used for. The lentivirus vector is increasingly used to integrate siRNA efficiently in a wide variety of cell lines and primary cells, both in vitro and in vivo.

### Rapid virus purification by Membrane Chromatography

The Sartobind® ion exchange membrane adsorber technology used in AdenoPACK and LentiSELECT is unique in its capability to efficiently and rapidly capture and recover large virus particles. When compared to chromatography media, membrane adsorbers provide large 3000 nm pores allowing unrestricted access and recovery of virus from the charged adsorber surface. Convective flow through the syringe filter devices provides high-speed separations not possible with traditional chromatography, cesium chloride density gradients and ultracentrifugation methods. Our membrane adsorbers with porous matrices, high capacities, low differential pressures, high flow rates and low unspecific adsorption show an excellent performance in small scale virus purification. Additionally, they are also scalable and confirm to cGMP facilities to large volume, high performance separation, reducing the processing time by a factor of 10 in the final process.



# Adenovirus Purification with Vivapure AdenoPACK kits

## AdenoPACK 20|100|500

The AdenoPACK adenovirus purification and concentration kits offer researchers who need to recover up to  $3 \times 10^{13}$  purified recombinant adenovirus particles for in-vitro transfection a fast, safe and easy to use solution. The kits include all reagents and devices necessary for clarification, purification and concentration of adenovirus type 5 from HEK293 cell cultures in only two hours. These straight forward kits replace time-consuming and labor-intensive 48 hour CsCl density gradients.

AdenoPACK kits are offered as AdenoPACK 20, AdenoPACK 100 and AdenoPACK 500, for the purification and concentration of adenovirus type 5 from 20 ml to 500 ml cell culture, leading to  $1 \times 10^{11}$ -  $3 \times 10^{13}$  purified viral particles. For each sample volume, the most convenient handling method is offered for ultimate convenience.

To this end, preparations using AdenoPACK 20 are pursued in spin column format in a centrifuge, AdenoPACK 100 is a manually operated kit in syringe filter format\*, and AdenoPACK 500 is a pump driven kit.

\* Vivapure® AdenoPACK 100 can optionally be operated with a laboratory pump and an infusion pump, for which protocols are provided on our web page [www.sartorius-stedim.com](http://www.sartorius-stedim.com). Additionally, the tubes and adaptors needed for these operation modes can be ordered.

## AdenoPACK advantages

### Fast and easy virus purification

- Purification completed in 2 hours
- Convenient, over 10 x faster alternative to CsCl density gradient

### Quantitative yields

- In contrast to CsCl density gradient, the complete cell culture is used for virus purification and not only the viral pellet

### Flexible product range

- Applicable from initial construct screening to large scale virus production

### Complete Kit

- Including filtration devices, AdenoPACK units for virus purification, Vivaspin and all buffers

### Low endotoxin levels

- High cell viability and infection rates due to endotoxin levels of  $< 0.025$  EU/ml

## Purification results from preparations with Ad5 GFP-constructs

Purification method	Process time	Eluate	Recovery***	Viral Particles
AdenoPACK 20 20 ml culture	1 hour	1 ml	65-70%	$1 \times 10^{11-12}$
AdenoPACK 100 60 ml culture	1-2 hours	1 ml	65%	$1-3 \times 10^{12}$
AdenoPACK 100 200 ml culture	2 hours	1 ml	80%	$1 \times 10^{13}$
AdenoPACK 500 500 ml culture	2 hours	1 ml	80%	$1-3 \times 10^{13}$
500 ml CsCl density gradient	24-48 hours	1-2 ml**	60-70%	$1 \times 10^{11-12}$

\*\* after dialysis

\*\*\* before buffer exchange

## Vivapure® AdenoPACK 20 – The optimal kit for construct screening



Vivapure® AdenoPACK 20 is the downscale kit in the AdenoPACK series, purifying up to  $1 \times 10^{12}$  adenovirus type 5 particles from 20 ml cell culture. Especially when testing new constructs, parallel and fast purifications of different adenoviruses are essential. This kit allows the rapid, simple and affordable spin column based purification of 6 different samples in parallel and

bridges a gap in the CsCl density gradient method – for the first time adenovirus type 5 can efficiently be purified from less than 100 ml cell culture volume!

### Typical Performance

For a normal yielding vector,  $1 \times 15$  cm culture plate purified using this method yields up to  $1 \times 10^{12}$  viral particles.

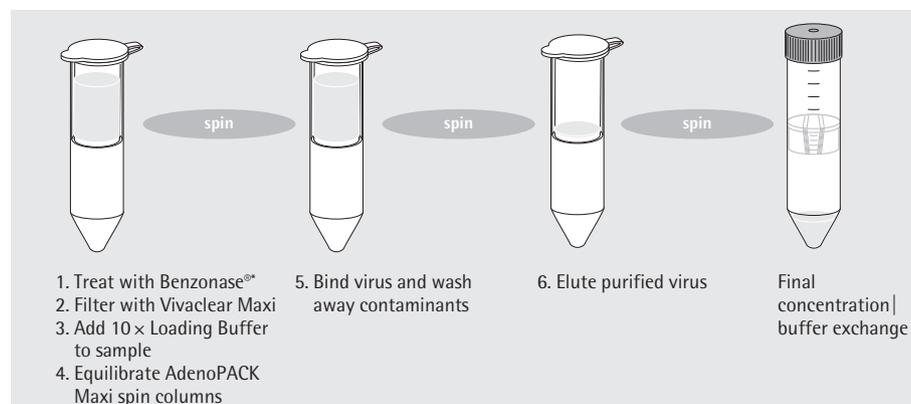
### Vivapure® AdenoPACK 20 contents and ordering information

	Sartorius Stedim Order No.	Scientific Fisher Order No.
Vivapure® AdenoPACK 20	VS-AVPQ020	14-558-548
AdenoPACK Maxi spin columns	6	
Vivaclear Maxi 0.45 µm PES	6	
Empty 50 ml tubes	6	
Loading Buffer (10×)	25 ml	
Washing Buffer (10×)	30 ml	
Elution Buffer	20 ml	
Benzonase® (12.5 U/µl)	120 µl	
Vivaspin 20, 100 kDa MWCO	6	
Instructions	1 each for Kit and Vivaspin	

### Technical Data

#### Kit Specifications

Sample size	20 ml of cell culture
Number of purifications	$6 \times 20$ ml
Virus particles (VP) per ml	Typically up to $1 \times 10^{11} - 10^{12}$
VP   IU	50–100
Processing time	Typically 1 hour
Endotoxin level	< 0.025 EU/ml



\* Benzonase® Nuclease is manufactured by Merck KGaA, Darmstadt, Germany and is covered by US Patent 5,173,418 and EP Patent 0,229,866. Nycomed Pharma A/S (Denmark) claims worldwide patent rights to Benzonase® Nuclease, which are licensed exclusively to Merck KGaA, Darmstadt, Germany. Benzonase® is a registered trademark of Merck KGaA, Darmstadt, Germany.

## Vivapure® AdenoPACK 100 – Fast purification of up to $1 \times 10^{13}$ viral particles



Vivapure® AdenoPACK 100 is optimally suited for adenovirus purification from up to 200 ml cell culture for in vitro transfection. This flexible kit contains two AdenoPACK 100 units, which can be either used in tandem for the purification of up to 200 ml cell culture for recovering  $1 \times 10^{13}$  viral particles or individually for purifying  $1-3 \times 10^{12}$  viral particles from up to 60 ml cell culture. The purification is pursued manually with a syringe optimally attached to a retort stand. However, for even more convenience, protocols are provided for optionally running the virus purification with a peristaltic pump or with an infusion pump, in addition to detailed instructions for a manual operation supplied with the kit. The accessories needed for the operation with a pump are supplied as individual products.

### Typical Performance

For a normal yielding vector,  $10 \times 15$  cm culture plate purified using this method yields up to  $1 \times 10^{13}$  viral particles.

### Vivapure® AdenoPACK 100 contents and ordering information

	Sartorius Stedim Order No.	Scientific Fisher Order No.
Vivapure® AdenoPACK 100	VS-AVPQ101	14-558-549
AdenoPACK 100 units	2	
Minisart Plus	4	
20 ml syringe	4	
Tubing set and one way valve	2	
10 ml syringe (elution)	2	
Loading Buffer (10×)	1 × 25 ml	
Washing Buffer	1 × 120 ml	
Elution Buffer	1 × 20 ml	
Benzonase® 12.5 U/μl	200 μl	
Vivaspin 20 concentrator	4	
Instructions	1 each for Kit and Vivaspin	

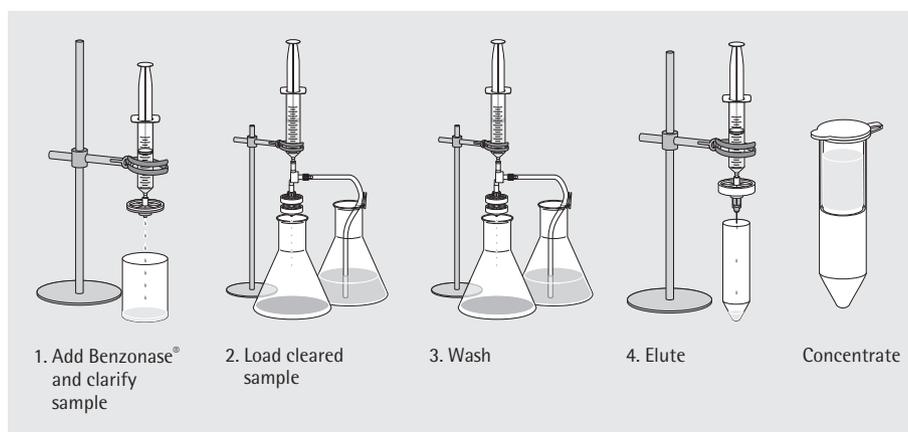
### AdenoPACK 100 Accessories

Pump tubing set for Vivapure AdenoPACK 100	VS-AVPA001	14-558-547
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## Technical Data

### Kit Specifications

Sample size	20–200 ml of cell culture
Number of purifications	2 × 20–60 ml 1 × 200 ml
Virus particles (VP) per ml	Typically up to $1 \times 10^{13}$
VP   IU	20–50
Processing time	Typically 2 hours
Endotoxin level	< 0.025 EU/ml



## Vivapure® AdenoPACK 500 – Pump driven Kit for larger volumes



Vivapure® AdenoPACK 500 is the direct upscale kit to the AdenoPACK 100, for adenovirus purification. In only 2 hours up to  $3 \times 10^{13}$  adenovirus particles are purified and concentrated from 500 ml cell culture. This completely ready-to-use kit is conveniently operated by a laboratory pump, offering optimal flow control and minimal hands-on time. This easy to use product replaces lengthy and inefficient cesium chloride density gradient methods.

### Typical Performance

For a normal yielding vector, 25 × 15 cm culture plate purified using this method yields up to  $3 \times 10^{13}$  viral particles.

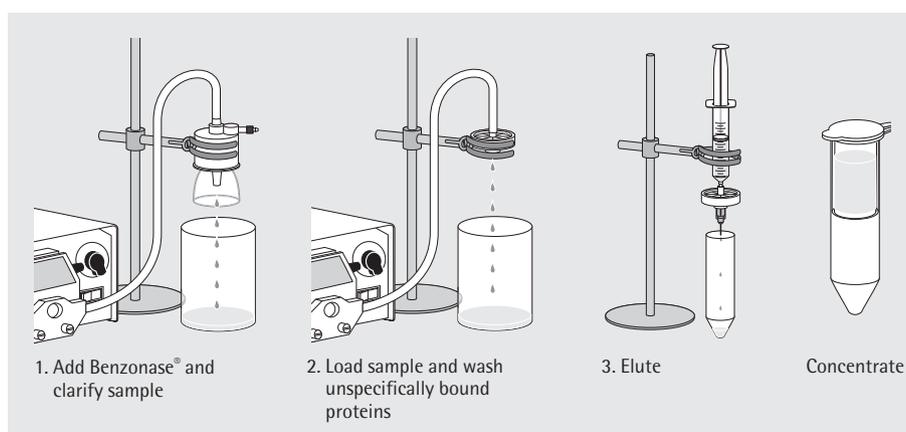
### Vivapure® AdenoPACK 500 contents and ordering information

	Sartorius Stedim Order No.	Scientific Fisher Order No.
Vivapure® AdenoPACK 500	VS-AVPQ501	14-558-550
AdenoPACK 500 unit	1	
Sartopore 2 150	1	
Tubing set and one way valve	2	
10 ml syringe	1	
Loading Buffer (10×)	60 ml	
Washing Buffer (10×)	30 ml	
Elution Buffer	20 ml	
Benzonase® 12.5 U/μl	500 μl	
Vivaspin 20 concentrator	2	
Instructions	1 each for Kit and Vivaspin	

## Technical Data

### Kit Specifications

Sample size	500 ml of cell culture
Number of purifications	1 × 500 ml
Virus particles (VP) per ml	Typically up to $3 \times 10^{13}$
VP   IU	20 – 50
Processing time	Typically 2 hours
Endotoxin level	< 0.025 EU/ml



## Lentivirus Purification with Vivapure® LentiSELECT kit

### LentiSELECT 40|500|1000

The LentiSELECT lentivirus purification and concentration kits offer researchers who need to recover up to  $5 \times 10^9$  infective lentivirus particles per ml for invitro transfection or animal studies a fast and easy to use solution.

These straight forward kits replace time-consuming ultracentrifugation protocols, which typically take approximately one day for large sample volumes, thus reducing the purification time to only a few hours.

LentiSELECT kits are offered as LentiSELECT 40, LentiSELECT 500 and LentiSELECT 1000 for the purification and concentration of VSV-G pseudotyped lentivirus from 40 ml to 1000 ml cell culture, leading to  $8 \times 10^8$  –  $1 \times 10^{10}$  purified infective particles. For each sample volume, the most convenient handling method is offered. To this end, 40 ml sample volumes are processed manually with LentiSELECT 40, while LentiSELECT 500 and 1000 are pump driven kits.

### LentiSELECT advantages

#### Fast and easy virus purification

- Purification completed in under one to six hours, depending on sample volume
- Kit as easy to use as filtration

#### No need for expensive instruments

- Lentivirus purification with LentiSELECT is independent of equipment such as ultracentrifuges

#### High virus purity

- Achieve pure virus due to a chromatography purification for your experiments instead of a crude and variable cell culture supernatant pellet

#### Optimal for multiple virus construct screening

- With LentiSELECT 40, four purification runs can be conducted in parallel with one kit

#### Complete Kits

- Including LentiSELECT units for virus purification, Vivaspins for concentration| buffer exchange and all buffers and syringes necessary

#### Low endotoxin levels

- High cell viability and infection rates due to endotoxin levels of  $< 0.025$  EU/ml

### Purification results from preparations with VSV-G pseudotyped lentivirus constructs

Purification method	Process time	Eluate	Viral Particles/ml	Recovery	Infective Viral Particles
LentiSELECT 40 40 ml sample	45 min	200 $\mu$ l*	$4 \times 10^9$	50%	$8 \times 10^8$
LentiSELECT 500 500 ml sample	3 hours	1 ml*	$3 \times 10^9$	35%	$2-5 \times 10^9$
LentiSELECT 1000 1000 ml sample	6 hours	2 ml*	$5 \times 10^9$	35%	$1 \times 10^{10}$
Ultracentrifugation   500 ml sample	10-11 hours	500 $\mu$ l	$6 \times 10^9$	25%	$3 \times 10^9$

\* After desalting | buffer exchange

## Vivapure® LentiSELECT 40 – Fast purification of up to $8 \times 10^8$ viral particles



Vivapure® LentiSELECT 40 is optimally suited for lentivirus purification for up to 40 ml cell culture and contains all components necessary for 4 purifications. Up to  $8 \times 10^8$  viral particles are recovered in less than one hour. In contrast to traditional ultracentrifugation methods, virus purification with Vivapure® LentiSELECT is fast and simple, without the need for expensive equipment like an ultracentrifuge. Addi-

tionally, this chromatographic procedure leads to pure virus samples in contrast to the crude ultracentrifuge pellet, resulting in higher reproducibility and increased gene transfer efficiency.

### Typical Performance

For a normal yielding vector,  $2 \times 15$  cm culture plate purified using this method yield up to  $8 \times 10^8$  particles.

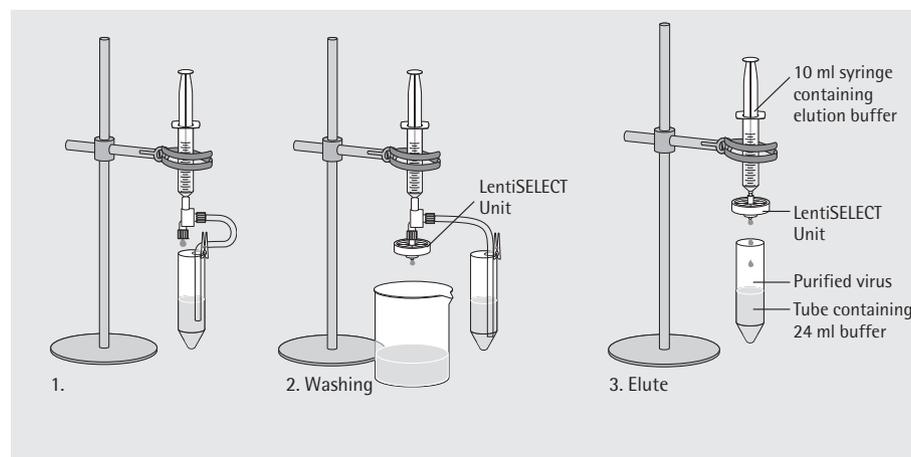
### Vivapure® LentiSELECT 40 contents and ordering information

	Sartorius Stedim Order No.	Scientific Fisher Order No.
Vivapure LentiSELECT 40	VS-LVPQ040	14-558-563
LentiSELECT units	4	
50 ml syringe	4	
10 ml syringe	4	
Tube set with one-way valve	4	
Loading buffer (10 ×)	30 ml	
Washing buffer	150 ml	
Elution buffer	20 ml	
Vivaspin 20, 100 kDa MWCO	8	
Instructions	1 each for Kit and Vivaspin	

### Technical Data

#### Kit Specifications

Sample size	40 ml cell culture
Number of purifications	$4 \times 40$ ml
Infective particles (P) per ml	Typically up to $3 \times 10^9$
VP   IU	5 – 15
Processing time	Typically 45 minutes
Endotoxin level	< 0.025 EU/ml



## Vivapure® LentiSELECT 500 – Fast purification of up to $2-5 \times 10^9$ infective particles per ml from 500 ml cell culture



Vivapure® LentiSELECT 500 is optimally suited for VSV-G pseudotyped lentivirus purification from up to 500 ml cell culture and contains all reagents and devices necessary for purifying up to  $2-5 \times 10^9$  infective particles.

The whole purification procedure is simply operated by a laboratory pump, which minimizes hands-on time. Unlike conventional purification methods as ultracentrifuga-

tion, Vivapure LentiSELECT 500 offers a fast and simple solution for purifying VSV-G pseudotyped lentiviruses making expensive purification equipment like ultracentrifuges redundant.

### Typical Performance

For a normal yielding vector, 500 ml cell culture purified using this method yield up to  $2-5 \times 10^9$  infective particles in 1 ml (total volume 1 ml).

### Vivapure® LentiSELECT 500 contents and ordering Information

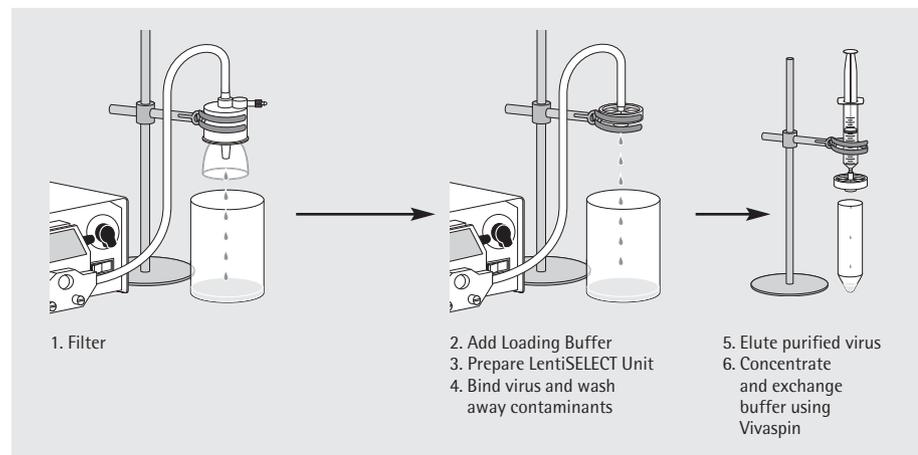
	Sartorius Stedim Order No.	Scientific Fisher Order No.
Vivapure® LentiSELECT 500	VS-LVPQ500	14-558-565
LentiSELECT unit	1	
Sartopore 2 150	1	
50 ml syringe	1	
Tube set with one-way valve	1	
Loading buffer (10 ×)	30 ml	
Washing buffer	170 ml	
Elution buffer	30 ml	
Vivaspin 20, 300 kDa MWCO	2	
Operating manual	1 each for Kit and Vivaspin	

### Technical Data

#### Kit Specifications

Sample size	500 ml cell culture
Number of purifications	1 × 500 ml
Infective particles (IP) per ml	Typically up to $2-5 \times 10^9$ *
Processing time	Typically up to 3 hours
Endotoxin level	< 0.025 EU/ml

\* 1 ml final elution sample



## Vivapure® LentiSELECT 1000 – Pump driven Kit for larger sample volumes



Vivapure® LentiSELECT 1000 is the direct scale up kit to LentiSELECT 500, for VSV-G pseudotyped lentivirus purification. The rapid 6 hour protocol results in a recovery of  $4-5 \times 10^9$  infective particles per ml (total volume 2 ml) from 1000 ml cell culture supernatant.

This kit is to be operated by a laboratory pump and contains all necessary buffers and ultrafiltration devices for optimal con-

venience. The traditional time consuming ultracentrifugation method is replaced by this fast and simple Vivapure LentiSELECT 1000 kit.

### Typical Performance

For a normal yielding vector, 1000 ml cell culture purified using this method yield up to  $4-5 \times 10^9$  infective particles in 1 ml (total volume 2 ml).

### Vivapure® LentiSELECT 1000 contents and ordering Information

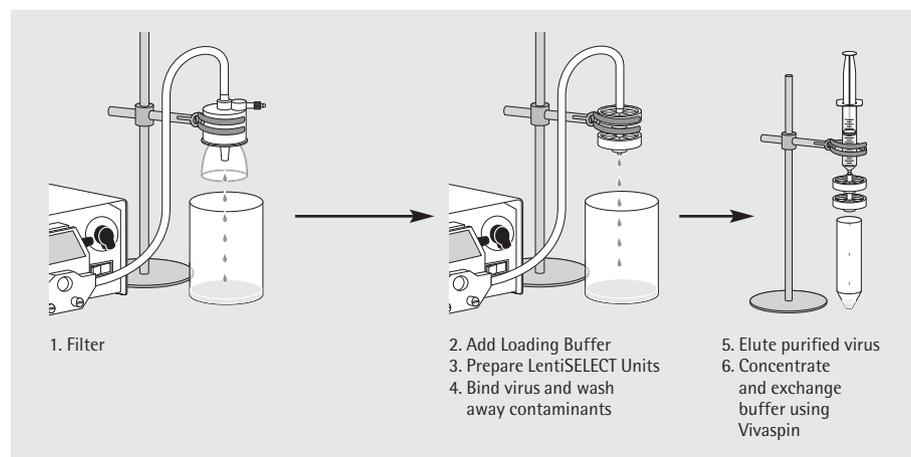
	Sartorius Stedim Order No.	Scientific Fisher Order No.
Vivapure® LentiSELECT 1000	VS-LVPQ1000	14-558-564
LentiSELECT unit	2	
Sartopore 2 150	1	
50 ml syringe	1	
Tube set with one-way valve	1	
Loading buffer (10 x)	30 ml	
Washing buffer	170 ml	
Elution buffer	60 ml	
Vivaspin 20, 300 kDa MWCO	2	
Operating manual	1 each for Kit and Vivaspin	

### Technical Data

#### Kit Specifications

Sample size	1000 ml cell culture
Number of purifications	1 × 1000 ml
Infective particles (IP) per ml	Typically up to $4-5 \times 10^9$ *
Processing time	Typically up to 6 hours
Endotoxin level	< 0.025 EU/ml

\* 2 ml final elution sample



# Application notes



## 1. Desalting and Buffer Exchange with Vivaspin Centrifugal Concentrators

### Introduction

Vivaspin centrifugal concentrators, with patented vertical membrane technology, combine fast filtration with high recovery of target proteins. This makes Vivaspin the technology of choice for desalting or buffer exchange, avoiding lengthy dialysis steps.

While proteins are retained by an appropriate ultrafiltration membrane, salts can pass freely through, independent of protein concentration or membrane MWCO. In consequence, the composition of the buffer in the flow-through and retentate is unchanged after protein concentration. By diluting the concentrate back to the original volume, the salt concentration is lowered. The concentrate can be diluted with water or salt-free buffer if simple desalting is required; however, it is also possible to dilute the concentrate with a new buffer, thereby exchanging the buffering substance entirely. For example, a 10 ml protein sample containing 500 mM salt, if concentrated 100x still contains 500 mM salt. If this concentrate is then diluted 100x with water or salt-free buffer, the protein concentration returns to normal, while the salt concentration is reduced 100x to only 5 mM, (I.E. a 99% reduction in salt).

The protein sample can then be concentrated again to the desired level, or the buffer exchange can be repeated to reduce the salt concentration even further before a final concentration of the protein. This process is called 'diafiltration'. For proteins with a tendency to precipitate at higher concentrations, it is possible to perform several diafiltration steps in sequence, with the protein concentrated each time to only 5 or 10x. For example, if a precipitous protein sample is concentrated to 5x then diluted back to the original volume, and this process is repeated a further two times, this still results in a >99% reduction in salt concentration, without over concentrating the protein.

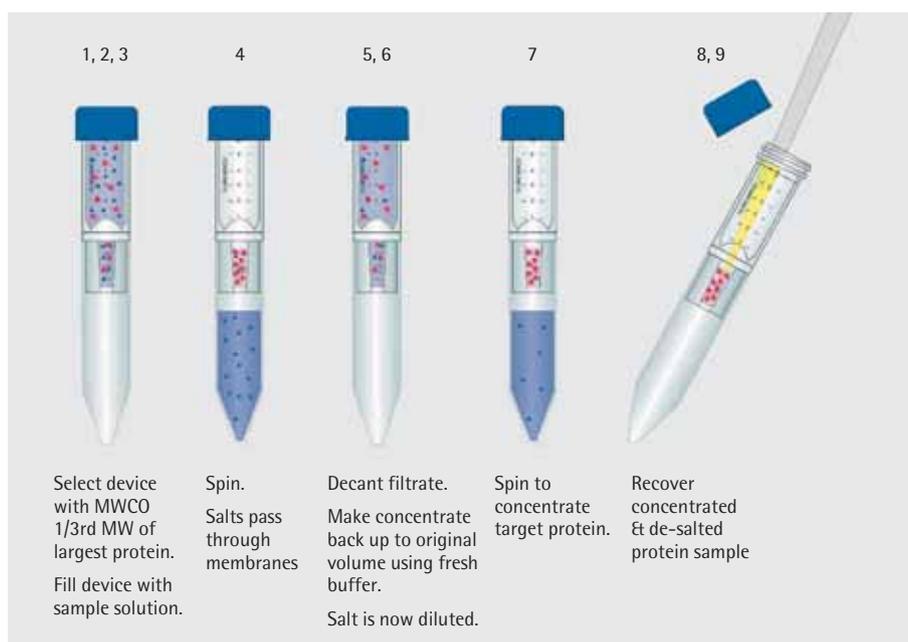


Figure 1: Step-by-step method for desalting and concentration

### Desalting and Buffer Exchange Procedure

(See Figure 1.)

1. Select the most appropriate MWCO for your sample. For maximum recovery, select a MWCO 1/2 to 1/3 the molecular size of the species of interest.
2. Fill concentrator with up to the maximum volume stated in the device operating instructions\*, (e.g. 20 ml if Vivaspin 20 is used).
3. If the sample is smaller than the maximum device volume\*, it can be diluted up to the maximum volume before the first centrifugation step. This will help increase the salt removal rate.
4. Centrifuge for the recommended amount of time at an appropriate spin speed for your Vivaspin model\*.
5. Empty filtrate container†.
6. Refill concentrator with an appropriate solvent.
7. Centrifuge again as before.
8. Empty filtrate container†.
9. Recover the concentrated, de-salted sample from the bottom of the concentrate pocket with a pipette.

#### Notes

\* For guidance on maximum fill volumes, spin speeds and suggested spin times, please refer to the Operating Instructions that accompany your Vivaspin products.

† Filtrate volumes should be retained until the concentrated sample has been analyzed.

### Test Results

As the results below show, the efficient design of Vivaspin devices allowed >95% of the salt to be removed during the first centrifugation step. Only one subsequent centrifugation step was needed to increase the typical salt removal to 99% with >92% recovery of the sample.

#### Vivaspin 20

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/ml		BSA 1 mg/ml		BSA 1 mg/ml		IgG 1 mg/ml	
	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal
Spin 1	100%	99%	97%	99%	97%	99%	90%	98%
Spin 2	96%	100%	92%	100%	93%	100%	87%	100%

Four Vivaspin 20 devices of each cut-off were tested with 20 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g. The devices > 5kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and second spin, the retentate was brought up to 20 ml with ultra pure water from the arium® system (Sartorius Stedim Biotech). OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with a Qcond 2200 conductivity measuring instrument.

#### Vivaspin 6

MWCO	5 kDa		30 kDa		50 kDa		100 kDa	
	Cytochrome C 0.25 mg/ml		BSA 1 mg/ml		BSA 1 mg/ml		IgG 1 mg/ml	
	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal	Protein Recovery	NaCl Removal
Spin 1	98%	99%	92%	99%	93%	99%	92%	98%
Spin 2	85%	100%	86%	100%	83%	100%	89%	100%

Four Vivaspin 6 devices of each cut-off were tested with 6 ml of solution. Each of the solutions contained 500 mM NaCl. Each spin was performed at 4,000 × g. The devices > 5 kDa were spun for 30 min. The devices with 5 kDa were spun 45 min. After the first and the second spin the retentate was brought up to 6 ml with ultra pure water from the arium® system (Sartorius Stedim Biotech) OD readings were taken at 410 nm for the Cytochrome C and 280 nm for the BSA and IgG samples. Salt concentration was measured with a Qcond 2200 conductivity measuring instrument.



## 2. Treatment of Vivaspin concentrators for improved recovery of low-concentrated protein samples

### Introduction

With appropriate device size and membrane cut-off selected, Vivaspin products will typically yield recoveries for the concentrated sample > 90% when the starting sample contains over 0.1 mg/ml protein of interest. Depending on sample characteristics relative to the membrane type used, solute (protein) adsorption on the membrane surface is typically very low (2–10 µg/cm<sup>2</sup>) and in practice not detectable.

This can increase to 20–100 µg/cm<sup>2</sup> when the filtrate is of interest and the sample must pass through the whole internal structure of the membrane. Whilst the relative adsorption to the plastic of the sample container will be proportionately less important than on the membrane, due to the higher total surface area, this can be also be a source of yield loss. Typically, a higher cut-off membrane will bind more than a low molecular weight alternative.

Whenever possible, the smallest MWCO and device size applicable should be chosen. Swinging bucket rotors are preferred to fixed angle rotors. This reduces the surface area of the concentrator that will be exposed to the solution during centrifugation.

An important factor not to be neglected is the thorough recovery of the retentate. Make sure to carefully remove all traces of solution from the sample container and, if feasible, rinse the device after recovering the sample with one or more drops of buffer and then recover again.

The intention of the following “passivation” procedure is to improve recovery of protein samples in the nano- to microgram concentration range by pretreating the device (membrane & plastic). For this purpose a range of solutions are suggested in Table 1.

Table 1: Passivation Solutions

Type	Concentration
Powdered milk	1% in arium® water
BSA	1% in PBS
Tween 20	5% in arium® water
SDS	5% in arium® water
Triton X-100	5% in arium® water
PEG 3000	5% in arium® water

### Passivation procedure for Vivaspin ultrafiltration concentrators

#### A) Passivation Procedure

1. Wash the concentrators once by filling with arium® water and spin the liquid through according to the respective protocol.
2. Remove residual water thoroughly by pipetting. **Caution: Take care not to damage the membrane with the pipette tip.**
3. Fill concentrators with the blocking solution of choice as given in Table 1.
4. Incubate the filled concentrators at room temperature for at least 2 hours (overnight is also possible except for **Triton X-100 which is not recommended for overnight incubation**).
5. Pour out the blocking solution.
6. Rinse the device 3–4x very thoroughly with arium® water and finally spin through.
7. The “passivated” devices are now ready for use. We recommend comparing different passivation reagents with an untreated device.

**Note**

It is necessary to rinse the device thoroughly before each washspin to ensure that traces of passivation compound are removed from the deadstop. Use the device immediately for protein concentration or store it at 4°C filled with arium® water, to prevent the membrane from drying.

**B) Evaluation of passivation effects (exemplary with BSA)**

1. Prepare a 10 µg/ml BSA stock solution e.g. by diluting 90 µl of the 4 mg/ml stock solution in 36 ml 0.1 M sodium borate pH 9.3. Mix well.
2. Fill Vivaspin 2 devices with 2 ml of this 10 µg/ml BSA solution and close with cap provided.
3. Spin the device in a swing-out rotor at 4,000 × g until the volume is to app. 100 µl.
4. Recover the concentrate and make back up to 2 ml with 0.1 M sodium borate pH 9.3
5. Determine recovered protein concentrations e.g. according to Bradford or BCA assays.

**Results and Discussion**

As an example, the effect of milk powder was analysed. It could be shown (Table 2) that the protein recovery of a 10 µg/ml BSA solution could be increased from around 70 to 90%. If milk powder is not interfering with sample purity and quality, it is a good starting point to improve recovery of diluted sample solutions.

**Protein recovery (10 µg/ml BSA) with Vivaspin PES 10 kDa after passivation**

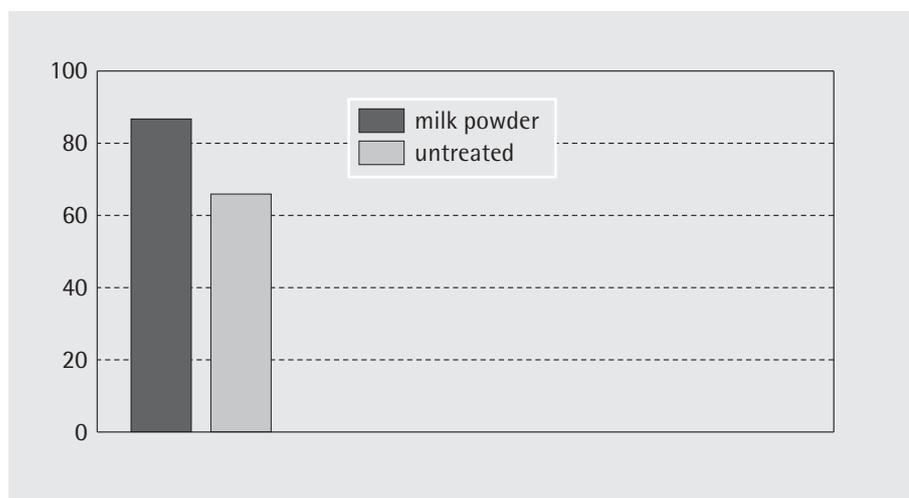
In another example, detergents were analysed with only 250 and 500 ng BSA (Table 3). BSA recovery declined to 50-30% in untreated devices as the protein concentration was reduced. Significant improvement to 60-90% recovery could be demonstrated when using the passivation strategy. Often, Triton X-100 seemed to work though the optimal reagent has to be selected for the respective protein and its hydrophilic | -phobic characteristics.

### Summary

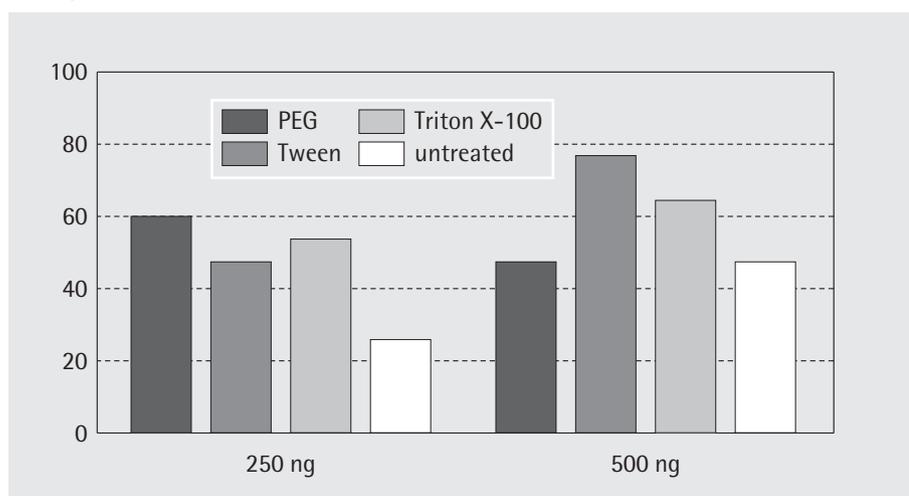
Passivation is an appropriate method to achieve increasing sample recovery when using very dilute samples. In addition to skimmed milk, other proteins (BSA), detergents and compounds are possible. However, it should be noted that this is a general procedure, not specific for any particular application. Depending on the hydrophilic|phobic character of the protein non-specific binding may be more or less of a problem and the suggested passivation solutions may lead to different results. Even with the Hydrosart membrane, which is recommend-

ed for dilute samples, passivation of the device will reduce losses on the plastic surface. One very important thing to remember is that the blocking agent is potentially introduced into the sample. It should be assured that this will not interfere with downstream analysis. For example, proteins must not be used for passivation if a pure protein is intended to be concentrated for x-ray crystallography, as even the smallest traces would interfere with the diffraction pattern. Other subsequent analyses methods include activity testing, gel electrophoresis or labelling are less problematic.

**Table 2: Protein recovery (10 µg/ml BSA) with Vivaspin PES 10 kDa after passivation**



**Table 3: Protein recovery (250 and 500 ng BSA) with Vivaspin 2 PES 10 kDa after passivation**





### 3. Scouting Protein Purification Conditions Using Vivapure® Centrifugal Ion Exchange Membrane Absorbers

#### Introduction

For separation and purification of proteins from biological samples, different characteristics of the target protein e.g. its size, charge, hydrophobicity or specifically engineered tags are exploited.

With ion exchange chromatography, separation is achieved on the basis of different charges of biomolecules. This makes it to a versatile method often used for pre-fractionation or purification of a target protein from crude protein mixtures. To optimize the purification procedure for an individual target, several binding and elution conditions have to be tested on cation and anion exchange matrices.

In contrast to traditional column chromatography methods, Vivapure® IEX centrifugal columns allow scouting of several chromatography conditions in parallel, leading quickly to different fractions which can be further analyzed for enriched or even already purified target protein.

Here, we demonstrate the performance of Vivapure® IEX Mini spin columns for evaluation of optimal purification conditions of cloned SH2 domains from an *E. coli* lysate in a two step procedure. This protocol can generally be employed for finding a purification method based on ion exchange chromatography for a given target protein as it is fast and only uses up small amounts of the sample.

In the first step of this protocol, binding conditions are evaluated by loading the sample on Vivapure® Q and S columns at various pH-values, eluting bound proteins with a high salt concentration buffer and analyzing all fractions for the target protein. This step results in the optimal binding pH and the best ion exchange chemistry for the purification.

In a second step, the best elution method is evaluated by applying increasing salt concentrations to columns which were shown to bind the target protein in step one, leading to a complete purification protocol in less than one hour.

#### Experiment

Using the described scouting procedure, a purification method for a SH2 domain expressed in *E. coli* was developed. In a first step, proteins were bound to the Vivapure® IEX membranes at different pH values, then eluted with high-salt buffer. In Step Two a fresh sample was adjusted to the respective pH elucidated previously as the best choice for binding the protein and was loaded onto a new column for refining optimal elution conditions.

#### Materials

- Vivapure® Mini Q H spin columns
- Vivapure® Mini S H spin columns
- Minisart syringe filter (0.45 µm CA, Sartorius Stedim Biotech GmbH)
- Centrifuge, 45°-fixed-angle rotor; 2000 × g

#### Buffers used

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Buffer A: 25 mM Citrate, pH 4

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Buffer B: 25 mM Potassiumphosphate, pH 6

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Buffer C: 25 mM HEPES, pH 8

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Buffer D: 25 mM Sodiumbicarbonate, pH 10

---

Buffer E: 25 mM Citrate, pH 4, supplemented with 1 M NaCl.

---

Buffer F: 25 mM Potassiumphosphate, pH 6, supplemented with 0.2 M, 0.4 mM, 0.6 mM, 0.8 mM, & 1 M NaCl, respectively.

---

Buffer G: 25 mM HEPES, pH 8, supplemented with 1 M NaCl

---

Buffer H: 25 mM Sodiumbicarbonate, pH 10, supplemented with 1 M NaCl

---

## Procedure

### Step One: Scouting for binding conditions to the appropriate ion exchange chemistry.

#### Expression of target protein

300 ml LB media were inoculated with 4 ml of an overnight culture and incubated at 37°C, shaking at 150 rpm until an OD<sub>600</sub> of 1.0 was reached. IPTG was added to a final concentration of 1 mM and incubated for further 4 h with shaking at 150 rpm. Cells were harvested by centrifugation at 4000 × g for 30 min at 4°C. The pellet was resuspended in 35 ml PBS (150 mM KPi, pH 7,3) and cells were lysed by addition of lysozyme to a final concentration of 0.1 mg/ml and incubation for 1 h at 37°C. Insoluble particles as cell debris were removed by centrifugation at 10000 × g for 30 min at 4°C.

#### Sample preparation

4 × 200 µl of the cell lysate were diluted with 1.8 ml binding buffer A to D each, to adjust the sample to the respective pH conditions. In order to avoid clogging of the membranes in the Vivapure® Mini spin columns, samples were clarified by passage through Minisart syringe filters.

#### Column equilibration

4 × Q and 4 × S Vivapure® Mini spin columns were labeled 4, 6, 8 and 10 corresponding to the pH of the buffer to be used. To each spin column, 400 µl of the corresponding binding buffer were added and spun for 5 minutes at 2000 × g.

#### Binding and washing

400 µl of the clarified samples adjusted to pH values 4, 6, 8 and 10 were applied each to the correspondingly equilibrated Vivapure® Q and S spin columns. Columns were spun for 5 min at 2000 × g. Afterwards, Vivapure® Mini spin columns were reloaded with 400 µl sample and spun again for 5 min at 2000 × g. Loosely bound proteins were washed away with the application of 400 µl of the respective binding buffer to each of the columns and spinning for 5 min at 2000 × g. Flow-through and wash fractions were collected for subsequent detection of the target protein.

#### Complete elution of bound proteins

200 µl of elution buffer E, F, G and H, were applied to the washed columns and spun for 3 min at 2000 × g. Eluates were saved for subsequent analysis.

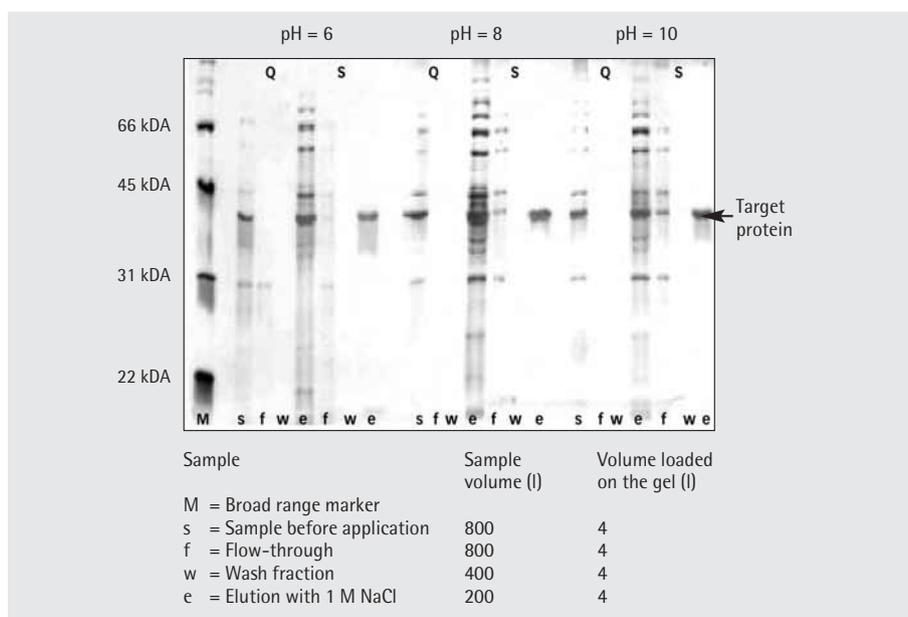


Fig. 1: Scouting for optimal binding conditions of a SH2 domain expressed in *E. coli*. SDS gel (reducing, 12%), silver stained. Shown are sample before loading, flow-through, wash, and elution fractions (1 M NaCl) from Vivapure® Q and S Mini spin columns, at the various pH values tested.

### Analysis

4  $\mu$ l of flow-through, wash, and elution fractions from each column were analyzed on reducing SDS-PAGE followed by silver staining.

### Result of Step One

Dilution of the *E. coli* lysate with binding buffer A (25 mM Citrate, pH 4) lead to complete precipitation of sample proteins. Thus, pH 4 could not be tested in this experiment. As can be seen on the SDS gel in figure 1, the target protein was present in the eluate of the Vivapure<sup>®</sup> Q Mini spin column at all pH values tested together with most of the *E. coli* proteins (Lanes Q "e"). In contrast, using the Vivapure<sup>®</sup> S Mini spin column, at all pH-values tested, most *E. coli* proteins did not bind to the membrane and were found in the flow-through (Lane Lane S "f"), thus resulting in pure target protein in all elution fractions (Lane S "e").

Differences could be detected in the binding efficiency of the target protein as at pH 8 traces of the target protein were already found in the flow-through, with slightly higher amounts at pH 10 (Lane S "e"). At pH 6, the most efficient binding of the target protein to the S membrane was observed. Now that the binding conditions, i. e. binding pH and the best suited ion exchange chemistry, were found, the elution protocol of the target protein was optimized in a second step.

### Step Two: Optimizing elution conditions

#### Sample preparation

Taking account of the results of Step One, 200  $\mu$ l cell lysate were diluted with 1.8 ml binding buffer B (25 mM KPi, pH 6). In order to avoid clogging of the membrane in the Vivapure<sup>®</sup> Mini spin column, the pH adjusted sample was clarified by passage through a Minisart syringe filter.

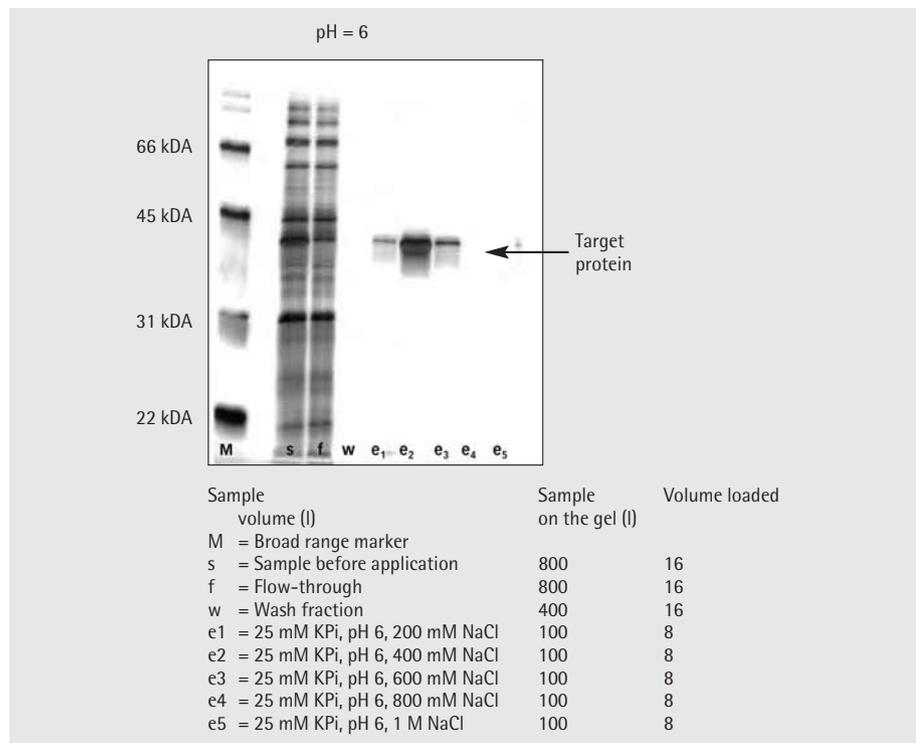


Fig. 2 Scouting for optimal elution conditions of a SH2 domain expressed in *E. coli*. SDS gel (reducing, 12%), silver stained. Sample before loading, flow-through, wash, and elution fractions from Vivapure<sup>®</sup> S Mini spin column at pH 6 are shown.

**Column equilibration**

400 µl binding buffer B were applied to one Vivapure® S Mini spin column and spun for 5 minutes at 2000 × g.

**Binding and washing**

400 µl of the clarified sample were applied to the equilibrated Vivapure® S column and spun for 5 min at 2000 × g. Afterwards, the Vivapure® S Mini spin column was reloaded with 400 µl sample and spun again for 5 min at 2000 × g.

Loosely bound proteins were washed away by application of 400 µl binding buffer to the column and spinning for 5 min at 2000 × g. Flow-through and wash fraction were saved for analysis.

**Stepwise elution**

100 µl elution buffer F, supplemented with 0.2 M NaCl were applied to the Vivapure® S Mini spin column and spun for 3 min at 2000 × g. The eluate was collected. In the next step, 100 µl of elution buffer F, supplemented with 0.4 M salt were applied and again spun for 3 min at 2000 × g. Elution was continued until the entire gradient had been tested, saving the eluates from each step.

**Analysis**

4 µl of flow-through, wash, and elution fractions from each column were analyzed on reducing SDS-PAGE followed by silver staining.

**Result of Step Two**

The target protein started to elute with 200 mM NaCl, however the main fraction eluted with 400 mM NaCl. Traces of the target protein were also found in the next elution step with 600 mM NaCl, but this might be due to the low elution volume.

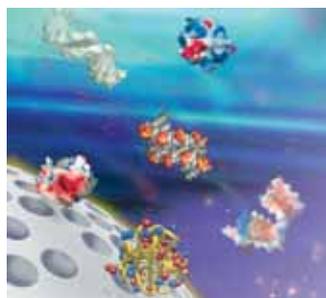
**Discussion**

A two-step procedure was used to rapidly scout optimal purification conditions for a target protein (a SH2 domain from *E. coli* lysate) with ion exchange chromatography. In the first step, the most suited buffer pH for binding the target protein to the most adequate ion exchanger was verified. In the second step, the elution condition was optimized building on the results gained in step one of this protocol (elution optimization after optimal binding of the target to the proper ion exchanger). With the scouting procedure described here, it was possible to quickly and conveniently purify the target protein to homogeneity. The results obtained in this experiment can be used for various ends, e.g:

- polishing a specific protein after a first chromatography step with another chemistry
- establishing quickly a FPLC method for a new protein
- finding a purification method for a new protein for upscaling with Vivapure® Maxi or Mega.

For these purposes Vivawell 96well plates, Vivapure® Maxi and Mega columns and Sartobind membrane adsorber units with FPLC connectors are available.

## 4. Removal of Endotoxin from Monoclonal Antibodies



**It is desirable to minimise endotoxins in purified protein preparations prior to their use in cell-based assays. Vivapure centrifugal anion exchange membrane devices can remove endotoxin from research grade monoclonal antibody solutions simply with high protein recovery.**

Endotoxins are lipopolysaccharides present in the cell wall of most Gram-negative bacteria, and are frequently present as contaminants in protein solutions purified in research environments. They have profound biological effects and thus must be minimised prior to use of such preparations in cell-based assays. The term EU is used to describe the activity of endotoxins, and typically the limit for endotoxin is set at 50 EU/mg for bioactive proteins destined for cell-based assays.

Achieving this low level is often a challenge in research as endotoxins are robust molecules surviving extremes of temperature and pH. Endotoxins are negatively charged under conditions commonly encountered during protein purification. This negative charge facilitates the use of anion exchange chromatography for their removal. If the binding of endotoxin can be achieved under conditions at which the protein of interest carries a net positive charge (i.e. at a pH below its isoelectric point) then the protein will be repelled from the positively charged matrix and flow through with the mobile phase, in what is often termed negative chromatography mode (Figure 1). However, this will often result in dilution of the protein, which may call for an additional concentration step.

Also, packing small chromatography columns and maintaining them sanitary is time consuming and requires specialist knowledge and equipment. Centrifugal ion exchange membrane spin columns offer an alternative to traditional chromatographic removal of endotoxin. They avoid the development of lengthy procedures with expensive equipment and potentially could rapidly yield high levels endotoxin-free protein.

In this report we tested the use of centrifugal anion exchange membrane devices for the removal of endotoxin from research grade antibody solutions.

### Absorption of endotoxin from a basic monoclonal antibody

#### Vivapure Mini Q spin columns

The monoclonal antibody used in this study has an isoelectric point of 7.5. All reagents and containers described below were supplied or prepared endotoxin free. Additionally, pH meter probes and magnetic stirrer bars were depyrogenated according to the manufacturers instruction or by soaking in 0.5 M sodium hydroxide for 1 hour. Vivapure Mini Q spin columns were washed sequentially with 0.5 ml of water for irrigation (WFI, Baxter), 0.5 ml of 0.5 M sodium hydroxide, 2 × 0.5 ml of WFI and 0.5 ml Dulbecco's phosphate buffered saline, pH 7.2 (PBS, Gibco) by loading each solution into the device followed by centrifugation at 2,000 × g for 5 minutes.

The monoclonal antibody (115 mg in 1.3 ml PBS) was divided equally amongst four mini spin columns and centrifuged as above. The flow through from each column was then filtered through a 0.2 µm sterilising centrifugal filtration device (Corning, Costar Spin-X, 2,000 xg for 5 minutes) and pooled.

Residual monoclonal antibody was recovered by washing each Vivapure mini column twice with 0.5 ml of phosphate buffered saline as above, collecting and combining the washes. Antibody concentration was measured in all samples using absorbance measurements at 280 nm and the known extinction coefficient. All volumes were estimated by weight assuming the density of the solutions to be 1 g/ml. Endotoxin (EU) was measured using a kinetic turbidimetric assay (Charles River Endosafe) following the manufacturers instructions.

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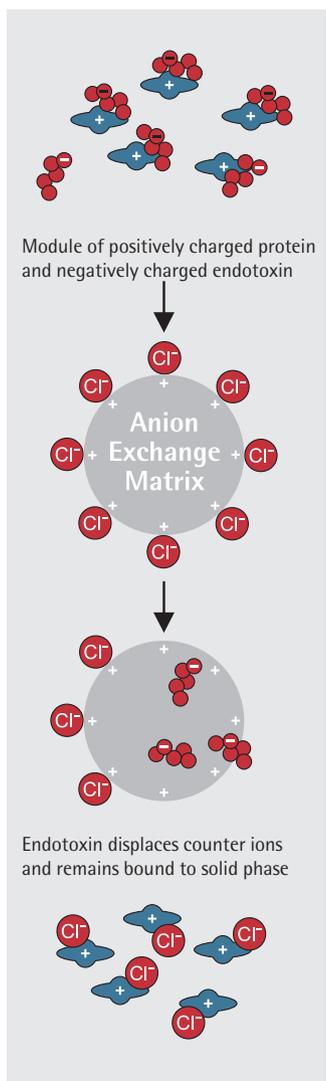


Fig. 1: Anion exchange of for endotoxin removal

**Vivapure Maxi H spin Q columns**

The monoclonal antibody used in this study has an isoelectric point of 6.0. Vivapure Maxi H spin Q columns were washed sequentially with 17 ml of water for irrigation (WFI, Baxter), 17 ml of 0.5 M sodium hydroxide and 3 × 17 ml of WFI and 17 ml Dulbecco's phosphate buffered saline, (Gibco, previously adjusted to pH 5.5 with the addition of concentrated hydrochloric acid) by loading each solution into the device followed by centrifugation at 500 × g for 5 minutes.

The monoclonal antibody (150 mg in 48 ml of PBS) was adjusted to pH 5.5 (i.e. below its pI) by the slow addition of dilute hydrochloric acid with constant mixing. This was then divided equally amongst four Vivapure maxi spin columns and centrifuged as above. The flow through from each column was then pooled and adjusted to pH 7.2 by the addition of 0.5 M sodium hydroxide. The pH-adjusted pool was then filtered through a 0.2 µm sterilising filter (Millipore Stericup or Vivascience Satorlab) and stored at 4°C. Residual monoclonal antibody was recovered from the Vivapure maxi columns by washing each with 15 ml of PBS adjusted to pH 5.5 as above, collecting and combining the washes. The concentration of monoclonal antibody and endotoxin levels in all samples was measured as described above.

**Results and discussion**

High recovery of antibody was achieved, for both the basic and acidic antibodies; 92% and 91% respectively (Tables 1 and 2). Very high clearance of endotoxin was also seen, with the levels being reduced to 1.2 and 1.3 EU/mg for both antibodies (Tables 1 and 2). The basic antibody product remained at constant concentration and was suitable for its intended use. The acidic antibody product was slightly reduced in concentration due to dilution on pH adjustment, but remained suitable for its intended use.

**Conclusions**

Vivapure centrifugal anion exchange membrane devices were effective in removal of endotoxin from research grade monoclonal antibody solutions. The clearance of endotoxin was maintained in a high conductivity buffer, PBS, preventing the need for any diafiltration into low salt buffers prior to the anion exchange. This method was also applicable to acidic proteins by simple pH adjustment prior to application to the charged membrane. In addition to the high protein recovery the starting concentration of the antibody solution was maintained obviating the need for any further processing. This method is a trouble-free method for reduction of endotoxin in protein solutions and would allow for easy processing of multiple samples over a short period.

**References**

Petsch, D. and Anspach, F.B. (2000) Endotoxin removal from protein solutions. *Journal of Biotechnology*, 76, 97–199.

**Table 1: Monoclonal antibody recovery and endotoxin level following purification using Vivapure Mini Q**

Sample	Total antibody (mg)	Antibody recovery (%)	Endotoxin (EU)
Start material	115	–	3450
Vivapure Mini Q Flow through	93	81	112
Vivapure Mini Q Wash #1	11	10	ND
Vivapure Mini Q Wash #2	1	1	ND

**Table 2: Monoclonal antibody recovery and endotoxin level following purification using Vivapure Maxi Q**

Sample	Total antibody (mg)	Antibody recovery (%)	Endotoxin (EU)
Start material	150	–	45,500
Vivapure Maxi Q Flow through	125	83	159
Vivapure Maxi Q Wash	12	8	ND

ND = Not determined







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