



# Lab Ultrafiltration and Purification Products

Simplifying Progress

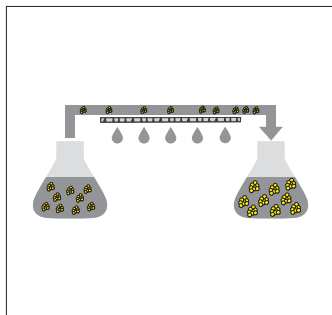
SARTORIUS

# Table of Contents

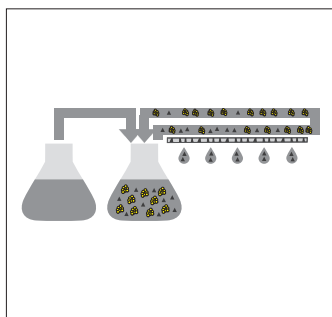
<b>General Information</b>	
Ultrafiltration Applications	4
Lab Ultrafiltration Devices	5
Ultrafiltration Process Methods	5
Membrane Performance Characteristics	6
Membrane Selection Guide	7
<b>Protein and Macromolecule Concentration</b>	<b>9</b>
Centrifugal and Pressurized Ultrafiltration	
▪ Vivaspin® 500	10
▪ Vivaspin® 2	12
▪ Vivaspin® Filtrate	14
▪ Vivaspin® Turbo 4 PES	16
▪ Vivaspin® 6	18
▪ Vivaspin® 15R	20
▪ Vivaspin® Endotest	22
▪ Vivaspin® Turbo 15 PES	24
▪ Vivaspin® Turbo 15 RC	26
▪ Vivaspin® 20	28
▪ Vivaspin® 100	30
▪ Vivaspin® Equipment and Accessories	32
Tangential Flow Filtration	
▪ Vivaflow® 50	34
▪ Vivaflow® 50R	36
▪ Vivaflow® 200	38
▪ Vivaflow® Equipment and Accessories	40
Ultrafiltration Membrane Discs	42

<b>DNA Concentration</b>	<b>45</b>
Vivacon® 500	46
Vivacon® 2	49
<b>Protein Purification</b>	<b>53</b>
Vivaclear Centrifugal Filters	54
Vivapure® Ion Exchange Purification Products	56
<b>Virus Purification and Concentration</b>	<b>59</b>
Vivapure® Virus Purification and Concentration Kits	60
Adenovirus Purification	61
▪ Vivapure® Adenopack 20	62
▪ Vivapure® Adenopack 100	63
▪ 5Vivapure® Adenopack 500	65
Lentivirus Purification	66
▪ Vivapure® Lentiselect 40	67
▪ Vivapure® Lentiselect 500	68
▪ Vivapure® Lentiselect 1000	69
<b>Application Notes</b>	<b>71</b>
1. Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators	72
2. Treatment of Vivaspin® Concentrators for Improved Recovery of Low-Concentrated Protein Samples	75
3. Scouting Protein Purification Conditions Using Vivapure® Centrifugal Ion Exchange Membrane Absorbers	78
4. Concentration and Purification of Viruses by using Ultrafiltration, Incl. Coronavirus - a Short Review	83
5. Sartorius Ultrafiltration Products in the Preparation of Biological Nanoparticles and Medical Nanocarriers	95
6. Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories	101

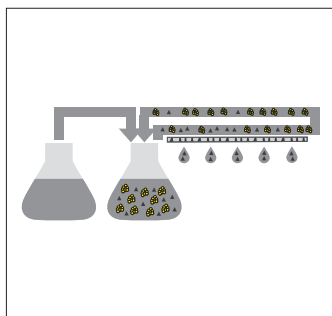
# Ultrafiltration Applications



Solute concentration



Solute fractionation



Solute desalting or purification

**Ultrafiltration is a convective process using 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 for purification or solvent exchange. Ultrafiltration is a gentle, non-denaturing method that is more efficient and flexible than alternative processes.**

## Typical Applications

- Concentration | desalting of proteins, enzymes, DNA, monoclonal antibodies, immunoglobulins, extracellular vesicles, viruses and nanoparticles
- Forensic DNA sample concentration prior to sequencing reaction
- Peptide fractionation in FASP (filter-aided sample preparation)
- Free drug | hormone assays
- Removal of primers from PCR amplified DNA
- Removal of labeled amino acids and nucleotides
- HPLC sample preparation
- Deproteinization of samples
- Recovery of biomolecules from cell culture supernatants | lysates
- Mammalian cell harvesting
- Cell washing, virus purification, cell debris removal and depyrogenation
- Environmental sample clarification | concentration

## Solute Concentration

Ultrafiltration membranes are used to increase the solute concentration of a desired biological or inorganic species. Macromolecules are retained by the membrane when they are significantly larger than the nominal pore size, while salts and microsolute are removed with the solvent.

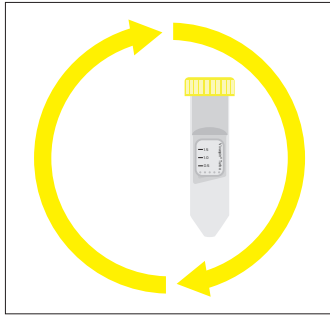
## Solute Fractionation

Ultrafiltration is a cost effective method for separating samples into size-graded components providing that the desired fractions have at least a 10-fold difference in molecular weight. During filtration, the permeating solute remains at its initial concentration whilst the retained macromolecules will be enriched.

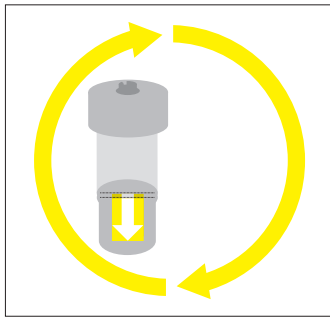
## Solute Desalting or Purification

A solution may be purified from salts, solvents and low molecular weight materials by diafiltration. Multiple solvent exchanges will progressively purify macromolecules from contaminating microsolute, which are typically removed most efficiently by adding solvent to the sample at a rate equal to the speed of filtration. This is called continuous diafiltration, and it replaces time-intensive techniques such as dialysis.

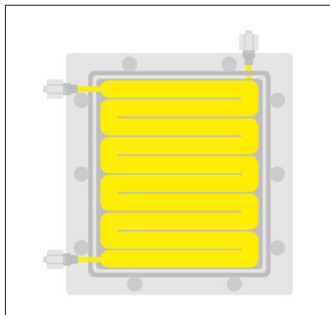
# Lab Ultrafiltration Devices



Centrifugal



Pressure and pressure-fugation



Crossflow | TFF

Sartorius develops devices dedicated to optimizing laboratory ultrafiltration processes with minimal time requirements while maximizing recovery, reliability and robustness.

In addition Sartorius are continually building technical and application

support tools to help users select the optimum device and process for their sample type.

Visit [www.sartorius.com](http://www.sartorius.com) for more technical and application support material.

## Ultrafiltration Process Methods

**Sartorius offers a comprehensive choice of operating methods for ultrafiltration and diafiltration. The guidance below will support you in selecting the most suitable method, depending on sample volume, equipment available, and the desired filtration speed and process control.**

### Centrifugal

**(0.1 to 90 mL starting volumes)**

Driven by centrifugal force, solvent and microsolute are cleared through the ultrafiltration membrane and into a filtrate container, usually positioned below. This gentle process is quick to set up and offers fast filtration speeds with most solutions. Twelve centrifugal devices are offered from the Vivaspin® and Vivacon® families.

### Pressure

**(5 to 98 mL starting volumes)**

Pressurized air or an inert gas provide the vector for ultrafiltration. For increased process speed, pressurized devices can be placed on an orbital shaker, where agitation impedes macromolecules from polarizing on the membrane surface. Vivaspin® 20 and 100 can be operated using gas pressure.

### Pressure-Fugation

**(5 to 15 mL starting volumes)**

A unique Sartorius method that combines gas pressure with centrifugation, with process times typically 30 to 50% faster than centrifugation alone. Vivaspin® 20 can be operated this way.

### Crossflow | TFF

**(0.1 to 5 L starting volumes)**

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 microsolute pass through the membrane into a separate filtrate vessel. Vivaflow® cassettes are offered for this method.

# Membrane Performance Characteristics

**Sartorius offers an extended range of membranes to cover the majority of ultrafiltration requirements. The following is a guide to selecting the most appropriate membranes according to their typical performance characteristics. However, membrane behavior and performance can be highly dependent on the specific characteristics of each sample. Therefore, it is recommended to experiment with multiple membrane materials when optimizing your ultrafiltration process.**

## **Polyethersulfone (PES)**

This is a low binding membrane that provides excellent performance with most solutions and exceptional recovery of negatively charged target molecules. Polyethersulfone membranes are usually preferred for their low fouling characteristics, exceptional flux and broad pH compatibility.

## **Regenerated Cellulose (RC)**

The Sartorius regenerated cellulose membrane has been uniquely developed to ensure optimal performance in the lab ultrafiltration devices.

This is a hydrophilic membrane suitable for general samples, with ultra-low protein adsorption and high chemical compatibility. Regenerated cellulose is especially well suited to ultrafiltration of oligonucleotides and peptides.

## **Hydrosart® (HY)**

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

## **Cellulose Triacetate (CTA)**

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

## **Membrane Performance Comparisons**

<b>Membrane</b>	<b>Frequently preferred for:</b>
Polyethersulfone & Regenerated Cellulose 3 kDa MWCO 5 kDa MWCO 10 kDa MWCO 30 kDa MWCO 50 kDa MWCO 100 kDa MWCO	Concentration Desalting Buffer exchange Fractionation
Cellulose triacetate 5 kDa MWCO 10 kDa MWCO 20 kDa MWCO	Deproteinization Free bound drug studies Whenever the filtrate is being analyzed
Hydrosart® 2 kDa MWCO 5 kDa MWCO 10 kDa MWCO 30 kDa MWCO	Concentration Desalting Buffer exchange Fractionation Membrane evaluation for scale up

# Membrane Selection Guide

**The advanced designs and low adsorption materials that characterize Sartorius ultrafilters, offer a unique combination of faster processing speeds and highest target molecule recoveries. Providing that the appropriate sample capacity, membrane material and MWCO are selected, these devices will typically yield recoveries in excess of 90% when the initial sample contains > 0.1 mg/mL of the solute of interest. The majority of any loss occurs through non-specific binding to the membrane surface and | or the sample container polymer.**

## 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 minimize this phenomenon by the selection of low binding 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 Optimization

When the highest recoveries are crucial, particularly when working with solute quantities in the microgram range, Sartorius recommends that users consider the following:

- Select the smallest device that suits the sample volume.
- Take advantage of the extra speed of Sartorius 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 recommended maximum.
- Avoid over-concentration. The smaller the final concentrate volume, the more difficult it is to achieve complete recovery.
- If feasible, after sample retrieval, rinse the device with one or more drops of buffer.
- Pretreat the device overnight with a passivation solution such as 5% SDS, Tween 20 or Triton X-100, then rinse thoroughly before use.

## Membrane Selection Guide (Recommended MWCO)

Application	< 5 kDa	10 kDa	30 kDa	50 kDa	100 kDa	> 300 kDa
Bacteria					■	■
Enzymes	■	■				
Extracellular vesicles					■	■
Growth factors	■	■				
IgG and mAbs			■	■	■	
Nucleic acids	■	■	■	■	■	
Oligonucleotides	■					
Peptides	■					
Viruses			■	■	■	■
Yeast					■	■

For highest recovery, select a membrane MWCO which is a maximum of one third to half the molecular weight of the solute to be retained



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VIVAFLOW 50

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# Protein and Macromolecule Concentration

## Table of Contents

Vivaspin® 500	10
Vivaspin® 2	12
Vivaspin® Filtrate	14
Vivaspin® Turbo 4 PES	16
Vivaspin® 6	18
Vivaspin® 15R	20
Vivaspin® Endotest	22
Vivaspin® Turbo 15 PES	24
Vivaspin® Turbo 15 RC	26
Vivaspin® 20	28
Vivaspin® 100	30
Vivaspin® Equipment and Accessories	32
Vivaflow® 50	34
Vivaflow® 50R	36
Vivaflow® 200	38
Vivaflow® Equipment and Accessories	40
Ultrafiltration Membrane Discs	42

# Vivaspin® 500



## 100 to 500 µL samples

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

The legacy patented vertical membrane design and thin channel filtration chamber (US 5,647,990), minimize membrane fouling and provide fast concentrations – even with particle-loaded solutions.

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	do not use
Fixed angle rotor	500 µL

### Dimensions

Length x diameter	50 x 11 mm
Active membrane area	0.5 cm <sup>2</sup>
Hold-up volume, membrane and support	< 5 µL
Dead-stop volume	5 µL

### Materials of construction

Body	Polycarbonate (PC)
Filtrate vessel	Polypropylene (PP)
Concentrator cap	Polycarbonate (PC)
Membrane	Polyethersulfone (PES)

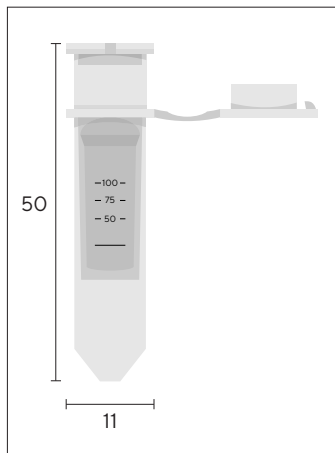
## Equipment Required

### Centrifuge

Rotor type	Fixed angle (min. 40°)
Rotor cavity	To fit 2.2 mL (11 mm) conical bottom tubes
Maximum RCF	12,000 g

### Concentrate recovery

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



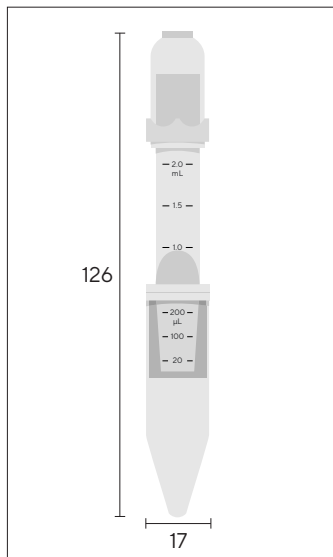
## Typical Performance Characteristics

	Time to concentrate up to 30x at 20°C and solute recovery	
Rotor	Fixed angle	
Centrifugal force	12,000 <i>g</i>	
Start volume	500 $\mu$ L	
	Time	Recovery
Aprotinin 0.25 mg/mL (6.5 kDa) 3 kDa MWCO PES	30 min	96%
BSA 1.0 mg/mL (66 kDa) 5 kDa MWCO PES	15 min	96%
10 kDa MWCO PES	5 min	96%
30 kDa MWCO PES	5 min	95%
IgG 0.25 mg/mL (160 kDa) 30 kDa MWCO PES	10 min	96%
50 kDa MWCO PES	10 min	96%
100 kDa MWCO PES	10 min	96%

## Ordering Information

Vivaspin® 500 PES	25 pc	100 pc
3 kDa MWCO	VS0191	VS0192
5 kDa MWCO	VS0111	VS0112
10 kDa MWCO	VS0101	VS0102
30 kDa MWCO	VS0121	VS0122
50 kDa MWCO	VS0131	VS0132
100 kDa MWCO	VS0141	VS0142
300 kDa MWCO	VS0151	VS0152
1,000 kDa MWCO	VS0161	VS0162
0.2 $\mu$ m	VS0171	VS0172

# Vivaspin® 2



## 0.4 to 3 mL samples

Vivaspin® 2 bridges the gap between the 500 µL and 4 mL centrifugal concentrators. This device combines the speed of the classic Vivaspin® products with low internal surface and membrane areas for superior recoveries from very dilute solutions.

Also unique to 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 concentrator recovery cap. Both methods result in near total concentrate recoveries.

Available with a choice of polyethersulfone, Hydrosart® or cellulose triacetate membranes, Vivaspin® 2 offers the highest flexibility for process optimization.

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	3 mL
Fixed angle rotor	2 mL

### Dimensions

Length x diameter	126 x 17 mm
Active membrane area	1.2 cm <sup>2</sup>
Hold-up volume, membrane	< 10 µL
Dead-stop volume	8 µL

### Materials of construction

Body	Polycarbonate (PC)
Filtrate vessel	Polycarbonate (PC)
Concentrator cap	Polycarbonate (PC)
Membrane	Polyethersulfone (PES) Hydrosart® (HY) Cellulose Triacetate (CTA)

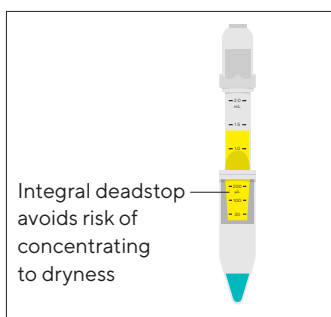
## Equipment Required

### Centrifuge

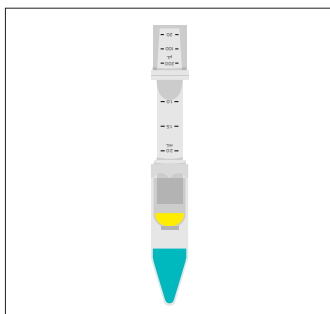
Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 15 mL (17 mm) conical bottom tubes	To fit 15 mL (17 mm) conical bottom tubes
Maximum RCF	4,000 g	8,000 g

### Concentrate recovery

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



PES, HY or CTA membranes



Reverse spin concentrate retrieval

## Typical Performance Characteristics

	Time to concentrate up to 30× at 20°C and solute recovery	
Rotor	Fixed angle	
Centrifugal force	5,000 g	
Start volume	2 mL	
	Time	Recovery
Insulin chain A 0.1 mg/mL (2.5 kDa) 2 kDa MWCO HY	35 min	95%
Aprotinin 0.25 mg/mL (6.5 kDa) 3 kDa MWCO PES	50 min	96%
BSA 1.0 mg/mL (66 kDa) 5 kDa MWCO PES	12 min	98%
5 kDa MWCO HY	22 min	98%
10 kDa MWCO PES	8 min	98%
10 kDa MWCO CTA	10 min	96%
10 kDa MWCO HY	12 min	98%
20 kDa MWCO CTA	5 min	96%
30 kDa MWCO PES	8 min	97%
30 kDa MWCO HY	5 min	97%
IgG 0.25 mg/mL (160 kDa) 20 kDa MWCO CTA	6 min	97%
30 kDa MWCO PES	10 min	96%
50 kDa MWCO PES	10 min	96%
100 kDa MWCO PES	8 min	95%

## Ordering Information

Vivaspin® 2 PES	25 pc	100 pc
3 kDa MWCO	VS0291	VS0292
5 kDa MWCO	VS0211	VS0212
10 kDa MWCO	VS0201	VS0202
30 kDa MWCO	VS0221	VS0222
50 kDa MWCO	VS0231	VS0232
100 kDa MWCO	VS0241	VS0242
300 kDa MWCO	VS0251	VS0252
1,000 kDa MWCO	VS0261	VS0262
0.2 µm	VS0271	VS0272
Vivaspin® 2 CTA		
10 kDa MWCO	VS02V1	VS02V2
20 kDa MWCO	VS02X1	VS02X2
Vivaspin® 2 HY		
2 kDa MWCO	VS02H91	VS02H92
5 kDa MWCO	VS02H11	VS02H12
10 kDa MWCO	VS02H01	VS02H02
30 kDa MWCO	VS02H21	VS02H22

# Vivaspin® Filtrate



## 0.5–2.5 mL samples

Vivaspin Filtrate® is a ready-to-use unit for low volume centrifugal ultrafiltration to separate proteins from low molecular weight substances in biological samples.

Vivaspin Filtrate® features a unique design that enables ultrafiltration in the direction opposite to 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 floating filtrate tube, where it is readily accessible without disassembly.

Vivaspin® Filtrate is ideal for the following applications:

- Drug binding studies
- Isolation of metabolites from serum
- Protein removal from blood samples
- Cleaning of liposomes
- Virus removal

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	2.5 mL
Fixed angle rotor	2.5 mL

### Dimensions

Length x diameter	93 x 14 mm
Active membrane area	0.79 cm <sup>2</sup>
Hold-up volume, membrane	< 5 µL
Dead-stop volume	100 µL

### Materials of construction

Centrifuge tube	Polystyrene (PS)
Filtrate tube	Styrene Acrylonitrile (SAN)
Concentrator cap	Polyethylene (PE)
Membrane	Cellulose Triacetate (CTA) Polyethersulfone (PES)

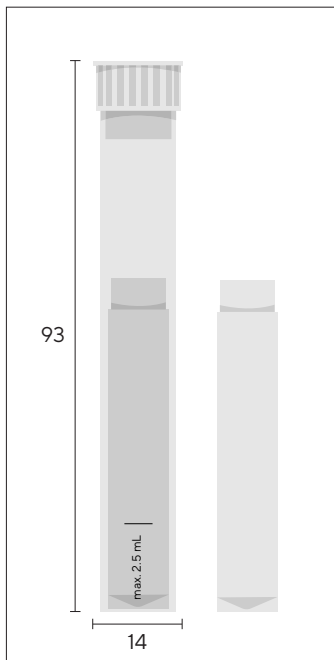
## Equipment Required

### Centrifuge

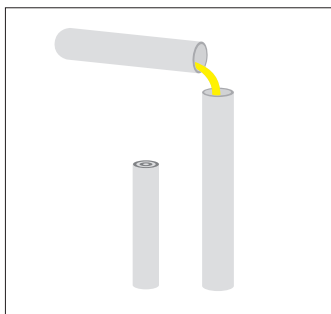
Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 15 mL (17 mm) conical   flat bottom tubes	To fit 15 mL (17 mm) conical   flat bottom tubes
Maximum RCF	2,500 g	2,000 g

### Concentrate recovery

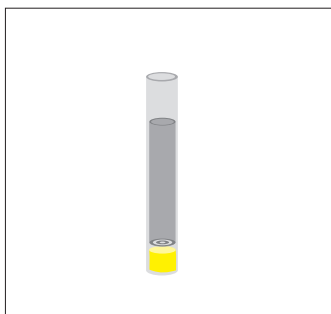
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type



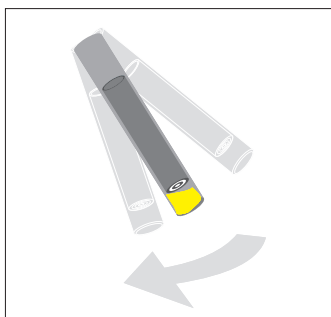
## Easy-to-use



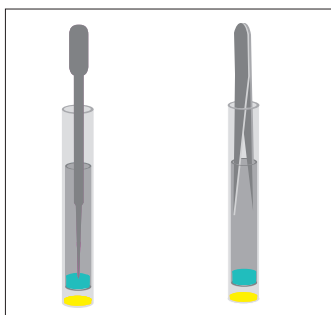
Remove filtrate tube, pour in sample



Replace filtrate tube



Centrifuge



Recover the filtrate...

...or use forceps to remove the filtrate tube and access the concentrate

## Typical Performance Characteristics

	Time to filter 50% of sample volume	Time to filter 90% of sample volume	Passage of sample species volume
Centrifugal force	2,000 g		
Start volume	2.5 mL		
BSA 1.0 mg/mL (66 kDa)			
5 kDa MWCO	300 min	–	0%
10 kDa MWCO	35 min	80 min	2%
20 kDa MWCO	9 min	20 min	2%
Blue Dextran 0.1 mg/mL (2,000 kDa)			
300 kDa MWCO	9 min	25 min	28%

## Ordering Information

<b>Vivaspin® Filtrate CTA</b>	<b>12 pc</b>
5 kDa MWCO	13229-E
10 kDa MWCO	13239-E
20 kDa MWCO	13249-E
<b>Vivaspin® Filtrate PES</b>	
300 kDa MWCO	13279-E

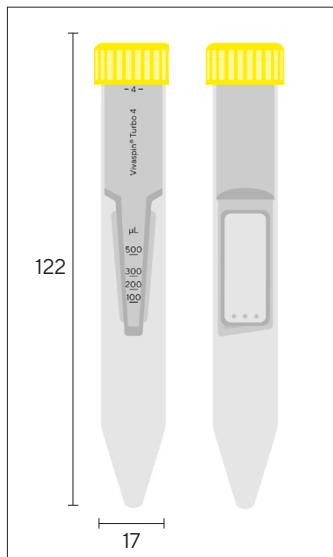
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R. H. Christenson, S. D. Studenberg, S. Beck-Davis and F. A. Sedor (1987). Digoxin-like immunoreactivity eliminated from serum by centrifugal ultrafiltration before fluorescence polarization immunoassay of digoxin. *Clinical Chemistry* **33**, 606-608

# Vivaspin® Turbo 4 PES



## 2 to 4 mL samples

Vivaspin® Turbo 4 PES offers the fastest sample concentration with the highest recoveries. This device can handle up to 4 mL sample volumes in swing bucket and fixed angle rotors that accept 15 mL conical bottom centrifuge tubes.

The optimized design and sleek internal profile ensure maximum process speeds all the way down to the last few microliters, resulting in more than 100-fold concentration.

UV joining technology provides a smooth transition between membrane and housing, allowing collection of the entire concentrated sample from the unique, pipette tip-friendly angular dead-stop pocket.

## Technical Specifications

Concentrator capacity	
Swing bucket rotor	4 mL
Fixed angle rotor	4 mL
Dimensions	
Length x diameter	122.5 x 17 mm
Active membrane area	3.2 cm <sup>2</sup>
Hold-up volume, membrane	< 10 µL
Dead-stop volume, swing bucket	40 µL
Dead-stop volume, fixed angle	30 µL
Materials of construction	
Body	Styrene Butadiene Copolymer (SBC)
Filtrate vessel	Polypropylene (PP)
Concentrator cap	Polypropylene (PP)
Membrane	Polyethersulfone (PES)

## Equipment Required

Centrifuge		
Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 15 mL (17 mm) conical bottom tubes	To fit 15 mL (17 mm) conical bottom tubes
Maximum RCF	4,000 g	7,500 g
Maximum RCF, 100 kDa MWCO	3,000 g	5,000 g
Concentrate recovery		
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type



## Visit us at

[www.sartorius.com/](http://www.sartorius.com/)

VivaspinTurbo4

for further information.

Here you can find instructions on how to use Vivaspin® Turbo 4 PES for:

- Desalting and buffer exchange
- Preparation of biological nanoparticles and medical nanocarriers
- Concentration and purification of viruses
- Urine protein concentration
- Separation of proteins and metabolites for disease detection

## Typical Performance Characteristics

	Time to concentrate up to 30× at 20°C and solute recovery			
	Swing bucket		Fixed angle (25°)	
Rotor	Swing bucket		Fixed angle (25°)	
Centrifugal force*	4,000 g		7,500 g	
Start volume	4 mL		4 mL	
	Time	Recovery	Time	Recovery
Cytochrome c (12.4 kDa)				
3 kDa MWCO PES	60 min	98%	80 min	96%
5 kDa MWCO PES	40 min	95%	50 min	94%
Lysozyme (14.3 kDa)				
3 kDa MWCO PES	65 min	95%	70 min	93%
5 kDa MWCO PES	50 min	94%	60 min	92%
α-Chymotrypsin (25 kDa)				
10 kDa MWCO PES	10 min	95%	8 min	95%
BSA (66 kDa)				
10 kDa MWCO PES	10 min	98%	7 min	97%
30 kDa MWCO PES	8 min	96%	6 min	97%
IgG (160 kDa)				
30 kDa MWCO PES	18 min	94%	13 min	92%
50 kDa MWCO PES	16 min	93%	12 min	90%
100 kDa MWCO PES	17 min	94%	13 min	92%

## Ordering Information

Vivaspin® Turbo 4 PES	25 pc	100 pc
3 kDa MWCO	VS04T91	VS04T92
5 kDa MWCO	VS04T11	VS04T12
10 kDa MWCO	VS04T01	VS04T02
30 kDa MWCO	VS04T21	VS04T22
50 kDa MWCO	VS04T31	VS04T32
100 kDa MWCO	VS04T41	VS04T42

\* 3,000 g (swing bucket) or 5,000 g (fixed angle) centrifugal force for 100 kDa MWCO devices.

# Vivaspin® 6

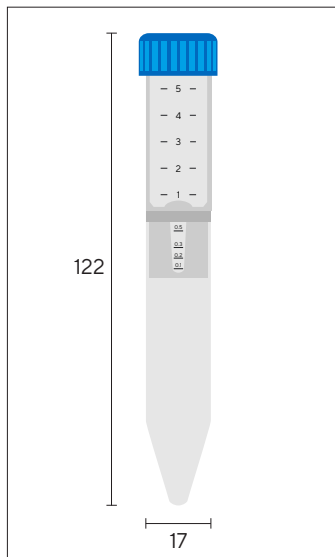


## 2 to 6 mL samples

Vivaspin® 6 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 centrifuge tubes.

Featuring twin vertical membranes for unparalleled filtration speeds and more than 100-fold concentration, the retentate volume is easily estimated from the printed graduations on the side of the concentrator. The modified dead-stop pocket further simplifies direct pipette retrieval of the final concentrate.



## Technical Specifications

Concentrator capacity	
Swing bucket rotor	6 mL
Fixed angle rotor	6 mL
Dimensions	
Length x diameter	122 x 17 mm
Active membrane area	2.5 cm <sup>2</sup>
Hold-up volume, membrane	< 10 µL
Dead-stop volume	30 µL
Materials of construction	
Body	Polycarbonate (PC)
Filtrate vessel	Polycarbonate (PC)
Concentrator cap	Polypropylene (PP)
Membrane	Polyethersulfone (PES)

## Equipment Required

Centrifuge		
Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 15 mL (17 mm) conical bottom tubes	To fit 15 mL (17 mm) conical bottom tubes
Maximum RCF	4,000 g	8,000 g
Maximum RCF, ≥100 kDa MWCO	4,000 g	6,000 g
Concentrate recovery		
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type

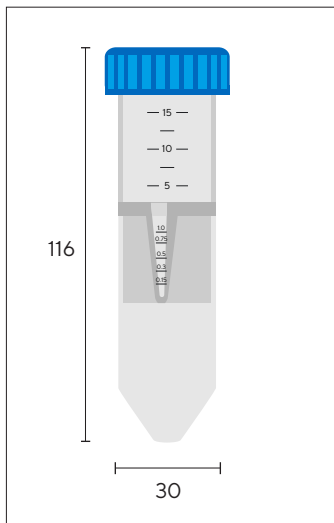
## Performance Characteristics

	Time to concentrate up to 30× at 20°C and solute recovery			
	Swing bucket		Fixed angle (25°)	
Rotor	Swing bucket		Fixed angle (25°)	
Centrifugal force	3,000 g		7,500 g	
Start volume	6 mL		6 mL	
	<b>Time</b>	<b>Recovery</b>	<b>Time</b>	<b>Recovery</b>
Cytochrome c 0.25 mg/mL (12.4 kDa) 3 kDa MWCO PES	-	-	90 min	97%
BSA 1.0 mg/mL (66 kDa) 5 kDa MWCO PES	20 min	98%	12 min	98%
10 kDa MWCO PES	13 min	98%	10 min	98%
30 kDa MWCO PES	12 min	98%	9 min	97%
IgG 0.25 mg/mL (160 kDa) 30 kDa MWCO PES	18 min	96%	15 min	95%
50 kDa MWCO PES	17 min	96%	14 min	95%
100 kDa MWCO PES	15 min	91%	12 min	91%
Latex beads 0.004% in DMEM + 10% FCS (55 nm) 300 kDa MWCO PES	-	-	25 min	99%
Latex beads 0.004% in DMEM + 10% FCS (240 nm) 1,000 kDa MWCO PES	-	-	4 min	99%
Yeast 1.0 mg/mL ( <i>S. Cerevisiae</i> ) 0.2 µm PES	4 min	97%	3 min	97%

## Ordering Information

Vivaspin® 6 PES	25 pc	100 pc
3 kDa MWCO	VS0691	VS0692
5 kDa MWCO	VS0611	VS0612
10 kDa MWCO	VS0601	VS0602
30 kDa MWCO	VS0621	VS0622
50 kDa MWCO	VS0631	VS0632
100 kDa MWCO	VS0641	VS0642
300 kDa MWCO	VS0651	VS0652
1,000 kDa MWCO	VS0661	VS0662
0.2 µm	VS0671	VS0672

# Vivaspin® 15R



## 2 to 15 mL samples

Vivaspin® 15R is designed for initial sample volumes up to 15 mL and features a modified regenerated cellulose membrane; Hydrosart®. This membrane is ideal where extremely high recovery with very low adsorption is needed. An example of this application includes desalting and concentration of immunoglobulin fractions.

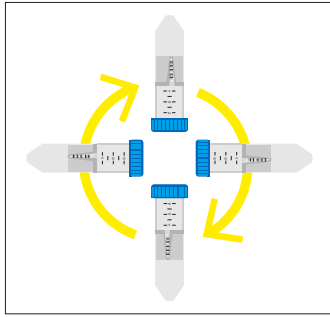
- Ultimate recoveries (95 – 98%)
- Extremely short concentration time (30-fold in 15 minutes)
- Simple and convenient handling
- Easy scale-up to 0.1 to 5 L with Vivaflow® 50R or 200 with Hydrosart® membranes
- Very low hold-up volume (< 20 µL)

## Technical Specifications

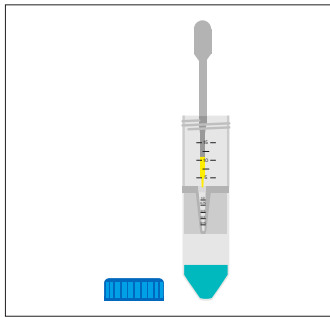
Concentrator capacity	
Swing bucket rotor	15 mL
Fixed angle rotor	12.5 mL
Dimensions	
Length x diameter	116 x 30 mm
Active membrane area	3.9 cm <sup>2</sup>
Hold-up volume, membrane	< 20 µL
Dead-stop volume	30 µL
Materials of construction	
Body	Polycarbonate (PC)
Filtrate vessel	Polycarbonate (PC)
Concentrator cap	Polypropylene (PP)
Membrane	Hydrosart® (HY)

## Equipment Required

Centrifuge		
Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 50 mL (30 mm) conical bottom tubes	To fit 50 mL (30 mm) conical bottom tubes
Maximum RCF	3,000 g	6,000 g
Concentrate recovery		
Pipette type	Fixed or variable volume	Fixed or variable volume
Recommended tip	Thin gel loader type	Thin gel loader type



Spin



Recover

## Typical Performance Characteristics

	Time to concentrate up to 30× at 20°C and solute recovery			
	Swing bucket		Fixed angle (25°)	
Rotor	3,000 g		6,000 g	
Centrifugal force	15 mL		12.5 mL	
Start volume	Time		Recovery	
Aprotinin 0.1 mg/mL* (6.5 kDa) 5 kDa MWCO	47 min	95%	45 min	95%
Cytochrome c 0.25 mg/mL* (12.4 kDa) 5 kDa MWCO	45 min	96%	45 min	96%
	25 min	94%	18 min	94%
α-chymotrypsin 0.25 mg/mL* (25 kDa) 5 kDa MWCO	50 min	98%	45 min	98%
	25 min	98%	18 min	98%
Ovalbumin 1.0 mg/mL* (45 kDa) 10 kDa MWCO	20 min	98%	14 min	98%
	15 min	94%	12 min	94%
BSA 1.0 mg/mL* (66 kDa) 30 kDa MWCO	18 min	98%	15 min	98%
	IgG 0.1 mg/mL in DMEM (160 kDa) 30 kDa MWCO	30 min	98%	25 min

## Ordering Information

Vivaspin® 15R HY	12 pc	48 pc
2 kDa MWCO	VS15RH91	VS15RH92
5 kDa MWCO	VS15RH11	VS15RH12
10 kDa MWCO	VS15RH01	VS15RH02
30 kDa MWCO	VS15RH21	VS15RH22

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

# Vivaspin® Endotest



## 2 to 15 mL samples

Vivaspin® Endotest is a single-use ultrafiltration device for endotoxin concentration and removal of interfering substances from liquid samples prior to LAL testing.

The centrifugal design enables parallel preparation of multiple test samples, minimizing hands-on time.

Vivaspin® Endotest can effectively be used in swing bucket or fixed angle rotors accepting 50 mL conical bottom centrifuge tubes.

These devices are certified pyrogen free ( $\leq 0.05$  EU/mL) and are available with 20 kDa MWCO cellulose triacetate membranes.

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	15 mL
Fixed angle rotor	12.5 mL

### Dimensions

Length x diameter	116 x 30 mm
Active membrane area	3.9 cm <sup>2</sup>
Hold-up volume, membrane	< 20 $\mu$ L
Dead-stop volume	30 $\mu$ L

### Materials of construction

Body	Polycarbonate (PC)
Filtrate vessel	Polycarbonate (PC)
Concentrator cap	Polypropylene (PP)
Membrane	Cellulose Triacetate (CTA)

## Equipment Required

### Centrifuge

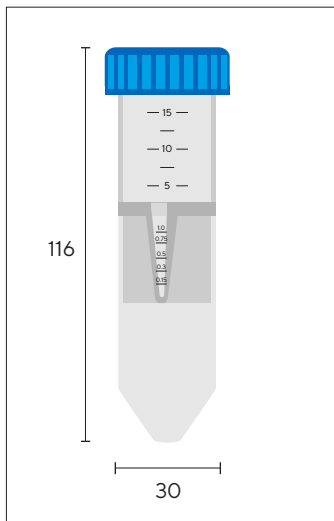
Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 50 mL (30 mm) conical bottom tubes	To fit 50 mL (30 mm) conical bottom tubes
Maximum RCF	1,000 g	1,000 g

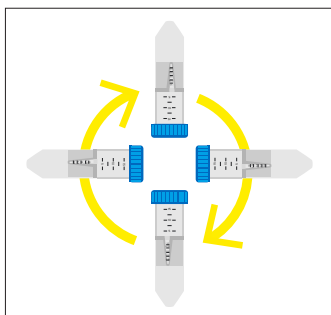
### Sample Mixing

Laboratory mixer	Vortex
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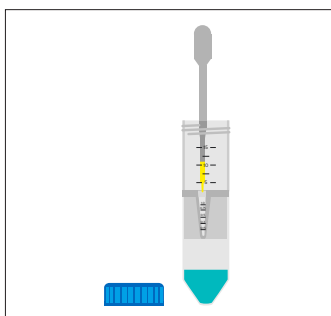
### Concentrate recovery

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





Spin



Recover

## Ordering Information

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Vivaspin® Endotest CTA

12 pc

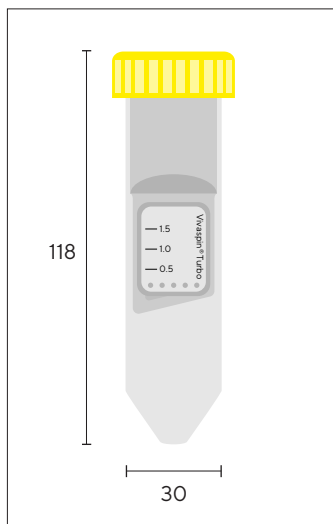
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20 kDa MWCO

VS15RXETO

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# Vivaspin® Turbo 15 PES



## 4 to 15 mL samples

Vivaspin® Turbo 15 PES enables the fastest sample concentration and highest recoveries. This device can handle samples up to 15 mL in rotors accepting 50 mL centrifuge tubes. The optimized design and sleek internal profile ensure maximum process speeds all the way down to the last few microlitres, resulting in more than 100-fold concentration.

UV joining technology provides a smooth transition between membrane and housing, allowing collection of the entire concentrated sample from the unique, angular dead-stop pocket.

Stable polyethersulfone membranes are suited to a wide pH range and especially recommended for high recovery of negatively charged target molecules. Now complemented with a regenerated cellulose option, Vivaspin® Turbo offers the best membrane, whatever the sample.

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	15 mL
Fixed angle rotor	11 mL

### Dimensions

Length x diameter	118 x 30 mm
Active membrane area	7.2 cm <sup>2</sup>
Hold-up volume, membrane	<10 µL
Dead-stop volume, swing bucket   fixed angle	100   60 µL

### Materials of construction

Body	Styrene Butadiene Copolymer (SBC)
Filtrate vessel	Polypropylene (PP)
Concentrator cap	Polypropylene (PP)
Membrane	Polyethersulfone (PES)

## Equipment Required

### Centrifuge

Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 15 mL (17 mm) conical bottom tubes	To fit 15 mL (17 mm) conical bottom tubes
Maximum RCF	4,000 g	4,000 g
Maximum RCF, 100 kDa MWCO	2,000 g	2,000 g

### Concentrate recovery

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



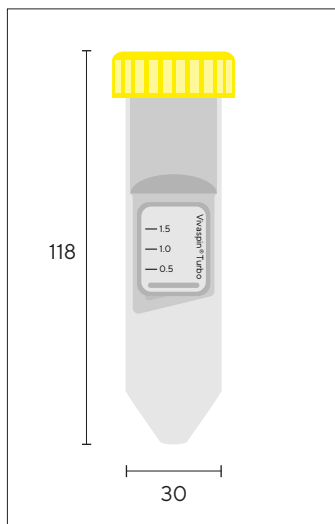
## Typical Performance Characteristics

	Time to concentrate up to 20× at 20°C and solute recovery			
	Swing bucket		Fixed angle (25°)	
Rotor	Swing bucket		Fixed angle (25°)	
Centrifugal force	4,000 g		4,000 g	
Start volume	15 mL		11 mL	
	Time	Recovery	Time	Recovery
Cytochrome c 0.25 mg/mL (12.4 kDa) 5 kDa MWCO PES	30 min	98%	50 min	98%
Lysozyme 0.25 mg/mL (14.3 kDa) 5 kDa MWCO PES	33 min	96%	50 min	96%
α-Chymotrypsin 1.0 mg/mL (25 kDa) 10 kDa MWCO PES	10 min	95%	10 min	95%
BSA 1.0 mg/mL (66 kDa) 10 kDa MWCO PES	10 min	99%	10 min	99%
30 kDa MWCO PES	8 min	98%	10 min	98%
IgG 1.0 mg/mL (160 kDa) 30 kDa MWCO PES	23 min	95%	17 min	95%

## Ordering Information

Vivaspin® Turbo 15 PES	12 pc	48 pc
3 kDa MWCO	VS15T91	VS15T92
5 kDa MWCO	VS15T11	VS15T12
10 kDa MWCO	VS15T01	VS15T02
30 kDa MWCO	VS15T21	VS15T22
50 kDa MWCO	VS15T31	VS15T32
100 kDa MWCO	VS15T41	VS15T42

# Vivaspin® Turbo 15 RC



## 4 to 15 mL samples

Vivaspin® Turbo 15 RC enables the fastest sample concentration and highest recoveries. This device can handle samples up to 15 mL in rotors accepting 50 mL centrifuge tubes. The optimized design and sleek internal profile ensure maximum process speeds all the way down to the last few microlitres, resulting in more than 100-fold concentration.

Solvent-free, heat weld technology provides a smooth transition between membrane and housing, allowing

complete concentrate recovery from the unique, angular dead-stop pocket.

Regenerated cellulose membranes developed specifically for Sartorius lab ultrafiltration devices are suited to general samples, with ultra-low adsorption and high chemical compatibility, and especially recommended for oligonucleotides and peptides. Complemented with a polyethersulfone option, Vivaspin® Turbo offers the best membrane, whatever the sample.

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	15 mL
Fixed angle rotor	11 mL

### Dimensions

Length x diameter	118 x 30 mm
Active membrane area	8.1 cm <sup>2</sup>
Hold-up volume, membrane	< 28 µL
Dead-stop volume, swing bucket   fixed angle	120   140 µL

### Materials of construction

Body	Styrene Butadiene Copolymer (SBC)
Filtrate vessel	Polypropylene (PP)
Concentrator cap	Polypropylene (PP)
Membrane	Regenerated Cellulose (RC)

## Equipment Required

### Centrifuge

Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 15 mL (17 mm) conical bottom tubes	To fit 15 mL (17 mm) conical bottom tubes
Maximum RCF	4,000 g	6,000 g
Maximum RCF, 100 kDa MWCO	3,000 g	6,000 g

### Concentrate recovery

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

## Typical Performance Characteristics

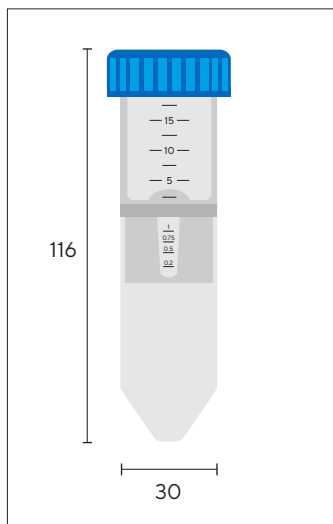
	Time to concentrate up to 20× at 20°C and solute recovery			
	Swing bucket		Fixed angle (25°)	
Rotor	Swing bucket		Fixed angle (25°)	
Centrifugal force	4,000 g*		6,000 g	
Start volume	15 mL		11 mL	
	<b>Time</b>	<b>Recovery</b>	<b>Time</b>	<b>Recovery</b>
Cytochrome c 0.25 mg/mL (12.4 kDa) 5 kDa MWCO RC	23 min	94%	37 min	92%
Lysozyme 0.25 mg/mL (14.3 kDa) 5 kDa MWCO RC	23 min	94%	37 min	89%
α-Chymotrypsin 1.0 mg/mL (25 kDa) 10 kDa MWCO RC	7 min	93%	9 min	92%
BSA 1.0 mg/mL (66 kDa) 10 kDa MWCO RC	8 min	94%	10 min	98%
30 kDa MWCO RC	4 min	96%	4 min	93%
IgG 1.0 mg/mL (160 kDa) 50 kDa MWCO RC	17 min	95%	11 min	96%
100 kDa MWCO RC	18 min	89%	12 min	89%

## Ordering Information

Vivaspin® Turbo 15 RC	12 pc	48 pc
5 kDa MWCO	VS15TR11	VS15TR12
10 kDa MWCO	VS15TR01	VS15TR02
30 kDa MWCO	VS15TR21	VS15TR22
50 kDa MWCO	VS15TR31	VS15TR32
100 kDa MWCO	VS15TR41	VS15TR42

\* 3,000 g for 100 kDa MWCO devices

# Vivaspin® 20



## 5 to 20 mL samples

Vivaspin® 20 centrifugal concentrators have been developed to offer increased sample and process flexibility and high performance.

Featuring twin vertical membranes for high filtration speeds, Vivaspin® 20 can achieve in excess of 100-fold concentrations factors.

The retentate volume is easily monitored using printed graduations and the modified dead-stop pocket simplifies direct retentate retrieval.

In addition, unique accessories are available for Vivaspin® 20, enabling pressurized, ultrafiltration and constant volume diafiltration.

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	20 mL
Fixed angle rotor	14 mL
With pressure head	15 mL

### Dimensions

Length x diameter	116 x 30 mm 125 x 30 mm with pressure head
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Active membrane area	6.0 cm <sup>2</sup>
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Hold-up volume, membrane	< 20 µL
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Dead-stop volume	50 µL
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### Materials of construction

Body	Polycarbonate (PC)
Filtrate vessel	Polycarbonate (PC)
Concentrator cap	Polypropylene (PP)
Pressure head	Polyoxymethylene (POM) and Aluminium (ALU)
Membrane	Polyethersulfone (PES)

## Equipment Required

### Centrifuge

Rotor type	Swing bucket	Fixed angle (min. 25°)
Rotor cavity	To fit 50 mL (30 mm) conical bottom tubes	To fit 50 mL (30 mm) conical bottom tubes
Maximum RCF	4,000 g	6,000 g
Maximum RCF, ≥100 kDa MWCO	3,000 g	6,000 g

### Pressure

Pressure accessories	VCA002, VCA005 and VCA200
Maximum pressure	5 bar (75 psi)
Maximum RCF, pressure-fuge*	3,000 g
Maximum RCF, pressure-fuge, ≥100 kDa MWCO*	2,000 g

\*Swing bucket only

## Equipment Required (Continued)

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

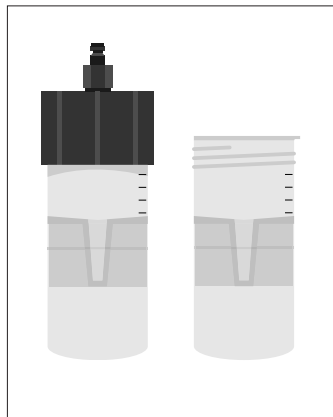
## Typical Performance Characteristics

Mode	Time to concentrate up to 30× at 20°C and solute recovery							
	Centrifuge		Centrifuge		Bench top		Press-fuge	
Rotor	Swing bucket		25° Fixed angle		Pressure		Swing bucket	
Centrifugal force   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 (12.4 kDa)								
3 kDa MWCO PES	110	97%	180	96%	60	96%	-	-
BSA 1.0 mg/mL (66 kDa)								
5 kDa MWCO PES	23	99%	29	99%	50	98%	14	98%
10 kDa MWCO PES	16	98%	17	98%	32	97%	8	97%
30 kDa MWCO PES	13	98%	15	98%	32	97%	8	97%
IgG 0.25 mg/mL (160 kDa)								
30 kDa MWCO PES	27	97%	20	95%	46	94%	13	97%
50 kDa MWCO PES	27	96%	22	95%	46	93%	13	96%
100 kDa MWCO PES	25	91%	20	90%	42	88%	12	94%
Latex beads 0.004% in DMEM +10% FCS (55 nm)								
300 kDa MWCO PES	20	99%	35	99%	10	99%	-	-
Latex beads 0.004% in DMEM +10% FCS (240 nm)								
1,000 kDa 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

Vivaspin® 20 PES	12 pc	48 pc
3 kDa MWCO	VS2091	VS2092
5 kDa MWCO	VS2011	VS2012
10 kDa MWCO	VS2001	VS2002
30 kDa MWCO	VS2021	VS2022
50 kDa MWCO	VS2031	VS2032
100 kDa MWCO	VS2041	VS2042
300 kDa MWCO	VS2051	VS2052
1,000 kDa MWCO	VS2061	VS2062
0.2 µm	VS2071	VS2072

# Vivaspin® 100



Device fits standard 250 mL rotors

## 20 to 98 mL samples

Vivaspin® 100 bridges the gap between centrifugal concentrators and crossflow cassettes. These devices feature vertical membranes for high speed processing of even high particle loaded samples. In addition, a unique choice between centrifugal, pressure or pressure-shake operating methods provides unrivaled process flexibility.

Fitting swing bucket rotors accepting 250 mL bottles, Vivaspin® 100 offers the highest sample capacity available in a centrifugal device – up to an astonishing 90 mL.

Vivaspin® 100 units can also be used for single or extremely sensitive samples of up to 98 mL when pressurized and left on the bench, or for temperature-sensitive samples, placed into a refrigerator. Pressurization is made easy by use of quick-release connectors and can be combined with orbital shaking for even faster sample concentration.

In whichever mode Vivaspin® 100 is used, the vertical membrane design inhibits membrane fouling while the integrated dead-stop impedes concentration to dryness and loss of sample.

## Technical Specifications

### Concentrator capacity

Swing bucket rotor	90 mL
With pressure head	98 mL

### Dimensions

Length x diameter	123 x 62 mm 197 x 62 mm with pressure head
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Active membrane area	23.5 cm <sup>2</sup>
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Hold-up volume of membrane	< 250 µL
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Dead-stop volume	350 µL
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### Materials of construction

Body	Polycarbonate (PC)
Filtrate vessel	Polycarbonate (PC)
Concentrator cap	Polypropylene (PP)
Pressure head	Polyoxymethylene (POM) and Aluminium (ALU)
Pressure head seal	Thermoplastic Elastomer (TPE)
Membrane	Polyethersulfone (PES)

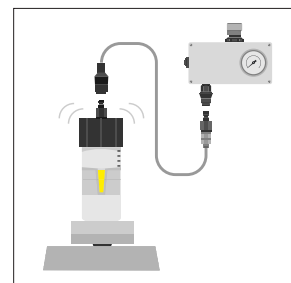
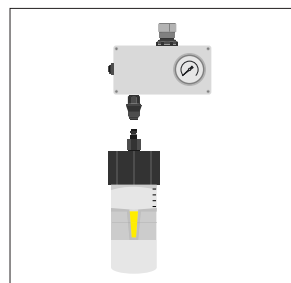
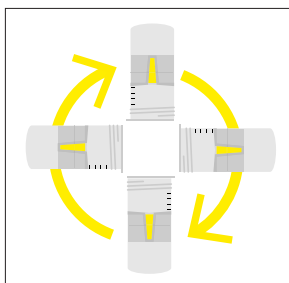
## Equipment Required

### Centrifuge

Rotor type	Swing bucket
Rotor cavity	To fit 250 mL (60 mm) centrifuge bottles (maximum cavity depth 105 mm)
Maximum RCF	2,000 g

### Pressure

Pressure accessories	VCA002, VCA800
Maximum pressure	5 bar (75 psi)



### Centrifuge

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

### Pressure

- Highest process control
- Use in fridge or cold room
- Slower concentrations

### Pressure-shake

- High process control
- Ideal for single samples
- Faster concentrations

## Typical Performance Characteristics

90 mL start volume	Time to concentrate up to 30× at 20°C			Solute recovery
	Swing bucket, 2,000 g	Pressure, 4 bar (60 psi)		
		Static	Orbital shake	
BSA 1.0 mg/mL (66 kDa)				
5 kDa MWCO PES	22 min	75 min	25 min	96%
10 kDa MWCO PES	16 min	60 min	20 min	96%
30 kDa MWCO PES	16 min	60 min	20 min	94%
IgG 0.25 mg/mL (160 kDa)				
50 kDa MWCO PES	20 min	70 min	30 min	94%
100 kDa MWCO PES	20 min	85 min	30 min	90%
Latex beads 0.004% in DMEM + 10% FCS (55 nm)				
300 kDa MWCO PES	35 min	-	120 min	99%
Latex beads 0.004% in DMEM + 10% FCS (240 nm)				
1,000 kDa MWCO* PES	4 min	5 min	4 min	99%

## Ordering Information

Vivaspin® 100 PES with PP cap	2 pc	10 pc
5 kDa MWCO	VC1011	VC1012
10 kDa MWCO	VC1001	VC1002
30 kDa MWCO	VC1021	VC1022
50 kDa MWCO	VC1031	VC1032
100 kDa MWCO	VC1041	VC1042
300 kDa MWCO	VC1051	VC1052
1,000 kDa MWCO	VC1061	VC1062
0.2 µm	VC1071	VC1072

## Vivaspin® Equipment and Accessories

### Gas Pressure Ultrafiltration

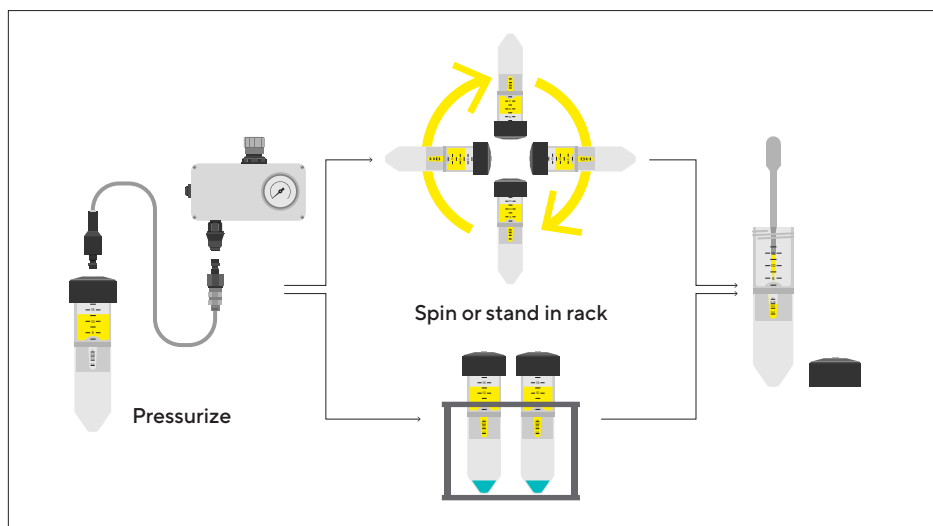
When an appropriate centrifuge is unavailable, or for single sample processing, Vivaspin® 20 and 100 centrifugal concentrators may be pressurized with compressed gas for bench-top concentration.

For even faster processing of samples in Vivaspin® 20, gas pressure can be combined with centrifugal force. This pressure-fugation method is particularly suitable for difficult to filter or viscous samples, such as serum, or when using low process temperatures, which reduce filtration speed, and generally when minimum process time is essential. In a similar way, Vivaspin® 100 may be pressurized and placed on an orbital shaker for faster processing.

### Constant Volume Diafiltration

In this procedure following concentration, a diafiltration cup inserted into the Vivaspin® 20 concentrator body is filled with buffer and centrifuged once to achieve 98% salt removal. This compares to the need for two centrifugation steps to achieve the same result with the re-fill and re-spin approach for discontinuous diafiltration.

The improved performance is due to the constant washing action of the exchange buffer from the diafiltration cup, as it replaces the original solvent and salts when they pass through the ultrafiltration membrane.



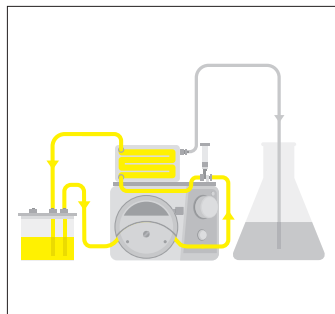
Using the Vivaspin® 20 pressure cap



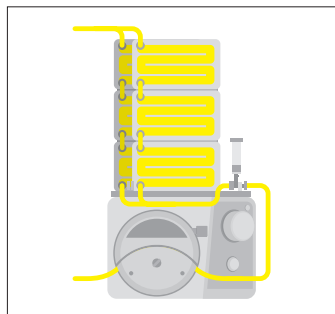
## Ordering Information

Vivaspin® Equipment and Accessories	Pack Size	Prod. No.
Air pressure controller (APC) fitted with pressure gauge, regulator, over-pressure safety valve and female coupling. APCs supplied with extension line (4 mm pneumatic tubing, 1 m) with male and female couplings, and inlet tubing (6 mm pneumatic tubing, 1 m)	1	VCA002
Charge valve for pressure head VCA200	1	VCA005
Female coupling	1	VCA010
Male coupling	1	VCA011
Replacement extension line (4 mm pneumatic tubing, 3 m)	1	VCA012
Vivaspin® 20 pressure head	1	VCA200
Vivaspin® 100 pressure head with seals	1	VCA800
Vivaspin® 100 pressure head seals	10	VCA014
Diafiltration cups	12	VSA005

# Vivaflow® 50



Single cassette



Multiple cassettes

## 0.1 to 3 L

The novel Vivaflow® 50 system 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 path provides high turbulence and cross flow velocities for exceptional flux, even at high concentrations.
- No need for pressure holders.
- Crystal clear for simple control and visibility of membrane status.
- Unique interlocking modules with series connectors for easy scale up.
- Disposable | single use.

### 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.

Each package of two cassettes contains all of the required tubing and fittings for plug-and-play operation with a standard peristaltic pump accepting 6.4 mm OD (size 16) tubing.

## Technical Specifications

### Dimensions

Overall L | W | H 25 | 107 | 84 mm

Channel W | H 15 mm | 0.3 mm

Active membrane area 50 cm<sup>2</sup>

Minimum recirculation volume < 10 mL

Hold-up volume, cassette 1.5 mL

Non recoverable hold-up < 0.5 mL

### Operating conditions

Pump flow rate 200 – 400 mL/min

Maximum pressure 3 bar (45 psi)

Maximum temperature 60°C

### Materials of construction

Main housing Polycarbonate (PC)

Flow channel Polymethylpentene (PMP)

Membrane Polyethersulfone (PES)  
Regenerated Cellulose (RC)

Membrane support Polymethylpentene (PMP)

Seals and O rings Silicone (SIL)

Flow restrictor Polypropylene (PP)

Fittings Polyamide (PA)

Tubing Polyvinyl Chloride (PVC), medical grade



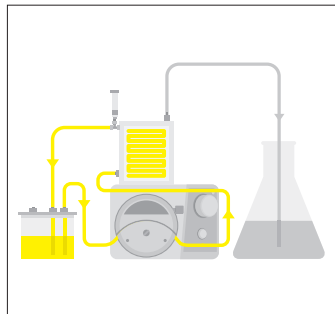
## Typical Performance Characteristics

	Time to concentrate up to 20x 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 kDa)				
5 kDa MWCO PES	34 min	49 min	96%	> 99%
10 kDa MWCO PES	22 min	32 min	94%	> 99%
30 kDa MWCO PES	22 min	32 min	92%	99%
50 kDa MWCO PES	20 min	29 min	92%	98%
IgG 1.0 mg/mL (160 kDa)				
100 kDa MWCO PES	43 min	62 min	92%	98%
100 kDa MWCO RC	40 min	58 min	92%	98%
Yeast 1.0 mg/mL ( <i>S. Cerevisiae</i> )				
0.2 µm PES	33 min	47 min	92%	98%

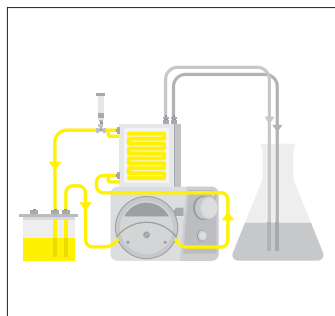
## Ordering Information

<b>Vivaflow® 50 PES</b>	<b>2 cassettes</b>
3 kDa MWCO	VF05P9
5 kDa MWCO	VF05P1
10 kDa MWCO	VF05P0
30 kDa MWCO	VF05P2
50 kDa MWCO	VF05P3
100 kDa MWCO	VF05P4
1,000 kDa MWCO	VF05P6
0.2 µm	VF05P7
<b>Vivaflow® 50 RC</b>	
100 kDa MWCO	VF05C4

# Vivaflow® 50R



Vivaflow® 50R - Single cassette



Vivaflow® 50R - Two cassettes

## 0.1 to 1 L samples

Concentrate 100 mL to under 20 mL in just a few minutes or concentrate one liter 50 times in less than 60 minutes. Alternatively, speed up your process by using two Vivaflow® 50R cassettes in parallel and concentrate 1 liters in under 30 min.

Vivaflow® 50R is a plug-and-play laboratory crossflow cassette for concentrating up to 1 L aqueous samples. The active membrane area per device is 50 cm<sup>2</sup>.

Each cassette is supplied with all the tubing and a pressure indicator for running the device with a laboratory pump and a size 16 pump head. For speeding up concentration, two cassettes can be run simultaneously.

- Fast and easy protein sample concentration
- Reusable
- Concentrates volumes from 0.1 L to 1 L
- Optimal for concentration of culture supernatants and viruses
- The most compact crossflow cassette with a premium Hydrosart® membrane

## Technical Specifications

### Dimensions

Overall L   W   H	24   100   100 mm
Channel W   H	7.5   0.4 mm
Active membrane area	50 cm <sup>2</sup>
Minimum recirculation volume	10 mL
Hold-up volume, cassette	1.7 mL
Non-recoverable hold-up	< 0.5 mL

### Operating conditions

Pump flow rate	200 - 400 mL/min
Maximum pressure	4 bar (60 psi)
Maximum temperature	60°C

### Materials of construction

Main housing	Acrylic
Flow channel	Acrylic
Membrane	Hydrosart® (HY)
Membrane support	Polyethylene (PE)
Seals and O-rings	Silicone (SIL)
Pressure indicator	Polypropylene (PP), SS spring
Flow restrictor	Polypropylene (PP)
Fittings	Polyamide (PA)
Tubing	Polyvinyl Chloride (PVC), medical grade

## Visit us at

[www.sartorius.com/Vivaflow50R](http://www.sartorius.com/Vivaflow50R)  
for further information.  
Here you can find instructions on how to use Vivaflow® 50R for:

- Preparation of biological nanoparticles and medical nanocarriers
- Concentration and purification of viruses

## Typical Performance Characteristics

	Time to concentrate up to 20× at 3.0 bar inlet   2.5 bar outlet pressure, 20°C			
	Start volume 250 mL	Average flux mL/min	Recovery	
			Direct	25 mL rinse
Lysozyme 0.25 mg/mL (14.3 kDa)				
5 kDa MWCO HY	70	3.4	96%	98%
10 kDa MWCO HY	23	10.3	94%	96%
BSA 1.0 mg/mL (66 kDa)				
10 kDa MWCO HY	24	9.9	98%	>99%
30 kDa MWCO HY	15	15.8	97%	>99%
IgG 1.0 mg/mL (160 kDa)				
100 kDa MWCO HY	46	5.2	97%	>99%
Start volume 1 L (one Vivaflow® 50R at 3 bar), BSA 1.0 mg/mL				
10 kDa MWCO HY	95	10.0	98%	>99%
Start volume 1 L (two Vivaflow® 50R in parallel at 3 bar), BSA 1.0 mg/mL				
10 kDa MWCO Hydrosart®	48	19.8	98%	>99%

## Ordering Information

Vivaflow® 50R HY	1 cassette
5 kDa MWCO	VF05H1
10 kDa MWCO	VF05H0
30 kDa MWCO	VF05H2
100 kDa MWCO	VF05H4

# Vivaflow® 200



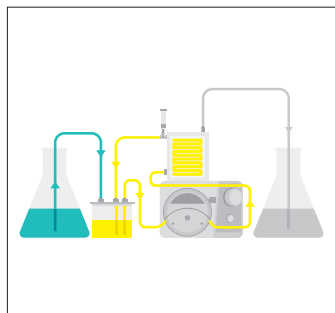
## 0.5 to 5 L

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 cassettes in parallel and concentrate 5 litres in under 75 minutes.

Each cassette is supplied complete with all required tubing and a pressure indicator. 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 tubing up to the standard hose barb connector.

Near total sample recoveries can be expected with most solutions.

Two cassettes in parallel will concentrate 5 litres in under 75 minutes.



Vivaflow® 200 set-up for diafiltration

## Technical Specifications

### Dimensions

Overall L | H | W 38 | 126 | 138 mm

Channel W | H 10 mm | 0.4 mm

Active membrane area 200 cm<sup>2</sup>

Minimum recirculation volume < 20 mL

Hold up volume, cassette 5.3 mL

Non-recoverable hold-up < 2 mL

### Operating conditions

Pump flow 200–400 mL/min

Maximum pressure 4 bar (60 psi)

Maximum temperature 60°C

### Materials of construction

Main housing Acrylic

Flow channel Acrylic

Membrane Polyethersulfone (PES)  
Hydrosart® (HY)

Membrane support Polypropylene

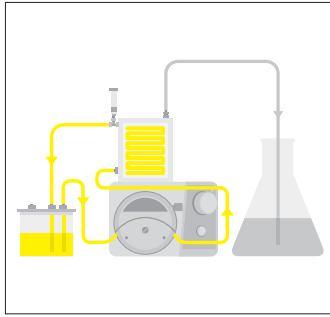
Seals and O rings Silicone (SIL)

Pressure indicator Polypropylene (PP), SS spring

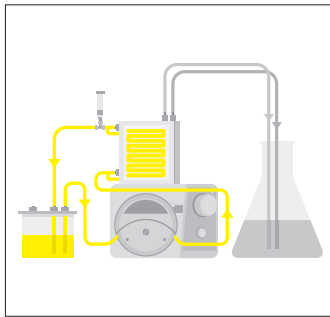
Flow restrictor Polypropylene (PP)

Fittings Polyamide (PA)

Tubing Polyvinyl Chloride (PVC), medical grade



Operation – Single cassette



Operation – Two cassettes

## Typical Performance Characteristics

	Time to concentrate up to 20× at 3 bar inlet pressure, 20°C			
	1 litre start volume	Average flux mL/min	Recovery	
			Direct	25 mL rinse
Lysozyme 0.25 mg/mL (14.3 kDa)				
2 kDa MWCO HY	160	6	97%	> 99%
3 kDa MWCO PES	180	5	97%	> 99%
BSA 1.0 mg/mL (66 kDa)				
5 kDa MWCO PES	29	33	98%	> 99%
5 kDa MWCO HY	70	14	98%	> 99%
10 kDa MWCO PES	23	41	96%	> 99%
10 kDa MWCO HY	35	27	98%	> 99%
30 kDa MWCO PES	25	38	96%	99%
30 kDa MWCO HY	20	48	96%	> 99%
50 kDa MWCO PES	22	43	96%	98%
IgG 1.0 mg/mL (160 kDa)				
100 kDa MWCO PES	54	18	96%	99%
Yeast 1.0 mg/mL ( <i>S. Cerevisiae</i> )				
0.2 µm PES	11	86	92%	98%
Dilute solute concentration, start volume 1 litre at 3 bar, 10 kDa MWCO PES				
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%
Start volume 5 litres (two VF200 in parallel at 3 bar) 10 kDa MWCO PES				
BSA 1.0 mg/mL (66 kDa)	67	70	97%	> 99%

## Ordering Information

<b>Vivaflow® 200 PES</b>	<b>1 cassette</b>
3 kDa MWCO	VF20P9
5 kDa MWCO	VF20P1
10 kDa MWCO	VF20P0
30 kDa MWCO	VF20P2
50 kDa MWCO	VF20P3
100 kDa MWCO	VF20P4
0.2 µm	VF20P7
<b>Vivaflow® 200 HY</b>	
2 kDa MWCO	VF20H9
5 kDa MWCO	VF20H1
10 kDa MWCO	VF20H0
30 kDa MWCO	VF20H2
100 kDa MWCO	VF20H4

# Vivaflow® Equipment



## Pumps and Complete Systems

Masterflex® peristaltic pump drives and heads perfectly complement Vivaflow® crossflow cassettes, while also offering the flexibility, durability and performance needed in multiple laboratory fluid transfer applications. With samples confined to single use tubing, there is no risk of cross-contamination and no cleaning required between each use.

Pump drives are offered for 230 or 115 V mains supplies. A variable speed, reversible motor enables easy sample

transfer, in with precise flow control in either direction. These pumps are also enabled for remote start | stop via a DB9 connector.

Loading tubing into the pump heads is fast and easy, thanks to the single-thumb operated lever and automatic tubing retention. Each model is compatible with multiple tubing sizes, extending their suitability to further applications, and the hard-wearing rollers ensure long-term durability.

## Technical Specifications

Dimensions	Masterflex® pump drives	Easy-Load pump heads
Length x width x height	230 x 183 x 134 mm	64 x 105 x 105 mm
Weight	4.1 kg	-
Power output	37 W	-
Speed   flow rate range	20 – 600 rpm	16 – 480 mL/min (size 16 tubing) 34 – 1,020 mL/min (size 15 tubing)
<b>Materials of construction</b>		
Housing	Polyarylamide (PARA)	Polyarylamide (PARA)
Rotor	-	Stainless Steel (SS) and PTFE
Rollers	-	Stainless Steel (SS)

## Ordering Information

Vivaflow® 50 System	Pack Size	Prod. No.
Pump drive (230 V), Easy-Load pump head (size 16), 500 mL diafiltration reservoir, cassette stand, spare tubing, T-connectors, series interconnectors and pressure indicator	1	VFS502
Pump drive (115 V), Easy-Load pump head (size 16), 500 mL diafiltration reservoir, cassette stand, spare tubing, T-connectors, series interconnectors and pressure indicator	1	VFS504
Vivaflow® 50R   200 System	Pack Size	Prod. No.
Pump drive (230 V), Easy-Load pump head (size 16), 500 mL diafiltration reservoir, and spare tubing	1	VFS202
Pump drive (115 V), Easy-Load pump head (size 16), 500 mL diafiltration reservoir, and spare tubing	1	VFS204
Pump Drives and Heads	Pack Size	Prod. No.
Masterflex® Economy Drive variable speed peristaltic pump (230 V)	1	VFP001
Masterflex® Economy Drive variable speed peristaltic pump (115 V)	1	VFP002
Masterflex® Easy-Load pump head (Size 16)	1	VFA012
Masterflex® Easy-Load pump head (Size 15)	1	VFA013



# Vivaflow® Accessories



## Scaling Up

The maximum throughput or speed of filtration with Vivaflow® cassettes can be easily increased, thanks to the modular design. Up to 6x Vivaflow® 50 cassettes can be operated in series and parallel, while two Vivaflow® 50R or 200 cassettes may be run in parallel.

## Accessories and Replacement Parts

Optional accessories such as the diafiltration reservoir, make concentration and diafiltration exceptionally convenient. A sample is

first concentrated to the desired volume, then a length of tubing placed into a separate vessel containing the exchange buffer is connected to the reservoir. Airtight sealing in the lid enables constant volume diafiltration. As the original buffer and salts permeate the ultrafiltration membrane, they are replaced by an equal volume of exchange buffer, thereby avoiding the large buffer volumes and possibility of sample dilution, which can be common to alternative methods.

## Technical Specifications

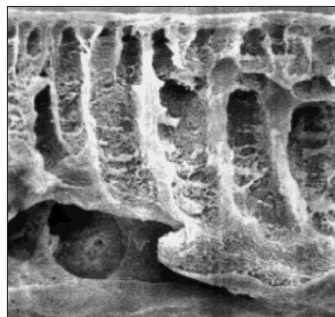
### Materials of construction

Cassette stand	Aluminium (ALU)
Tubing	Polyvinyl Chloride (PVC), medical grade
Luer fittings	Polyamide (PA)
Flow restrictors	Polypropylene (PP)
Pressure indicator	Polypropylene (PP), Stainless Steel (SS), Polyoxymethylene (POM), Silicone (SIL) and Polyamide (PA)
Diafiltration reservoir	Polycarbonate (PC), Polyoxymethylene (POM), Silicone (SIL), Polyvinyl Chloride (PVC) and Polyamide (PA)

## Ordering Information

Vivaflow® accessories for operating multiple cassettes	Pack Size	Prod. No.
T-connector (for 2-6x Vivaflow® 50   2x Vivaflow® 50R)	2	VFA030
Series interconnector (for 2-6x Vivaflow® 50)	6	VFA031
Y-connector (size 15 to 2x size 16, for 2x Vivaflow® 200)	1	VFA005
Vivaflow® accessories and replacement parts		
Vivaflow® 50 cassette stand	1	VFA016
500 mL diafiltration reservoir	1	VFA006
Pump tubing, 3 m, with luer fittings (size 16)	1	VFA004
Pump tubing, 3 m, with luer fittings (size 15)	1	VFA003
Female Luer lock to hose barb fittings (size 16)	10	VFA032
Female Luer lock to hose barb fittings (size 15)	10	VFA036
Flow restrictors, 0.6 mm	6	VFA035
Flow restrictors, 0.4, 0.6 and 0.8 mm (2 of each)	6	VFA009
Pressure indicator (1-3 bar)	1	VFA020
Vivaflow® tubing set (2x 1 m feed tubing with Luer fitting, 2x 0.5 m retentate tubing with flow restrictor, 1x series interconnector)	1	VFA034

# Ultrafiltration Membrane Discs



## Polyethersulfone (PES)

This is a general purpose membrane that provides excellent performance with most solutions when high recoveries in the retentate are of primary importance. Polyethersulfone membranes exhibit no hydrophobic or hydrophilic interactions and are usually preferred for their low fouling characteristics, exceptional flux and broad pH compatibility.

## Cellulose Triacetate (CTA)

High hydrophilicity and very low non-specific binding characterize this membrane. Cast without any

membrane support that could trap or bind passing microsolute, these membranes are preferred for sample cleaning, protein removal, and when high recoveries from the filtrate are of primary importance.

## Hydrosart® (HY)

These membranes are also highly hydrophilic and are often preferred for their higher protein recovery when processing very dilute solutions. Resistance to autoclaving, ease of cleaning and extended chemical compatibility also characterize this membrane material.

## Technical Specifications and Typical Performance

	Polyethersulfone, Type 146	Cellulose Triacetate, Type 145	Hydrosart®, Type 144
Thickness	120 µm	120 µm	180 µm
pH range	1 – 14	4 – 8	1 – 13
<b>Flux with water</b>			
10 kDa MWCO	0.2 mL/min/cm <sup>2</sup>	0.11 mL/min/cm <sup>2</sup>	0.08 mL/min/cm <sup>2</sup>
<b>Protein retention</b>			
Cytochrome c	95%	90%	99%

## Ordering Information

PES Membrane Discs, Type 146	Diameter	10 pc
1 kDa MWCO	47 mm	14609--47-----D
	63 mm	14609--63-----D
	76 mm	14609--76-----D
5 kDa MWCO	25 mm	14629--25-----D
	47 mm	14629--47-----D
	63 mm	14629--63-----D
10 kDa MWCO	76 mm	14629--76-----D
	25 mm	14639--25-----D
	43 mm	14639--43-----D
	47 mm	14639--47-----D
	63 mm	14639--63-----D
	76 mm	14639--76-----D
	150 mm	14639-150-----D

<b>PES Membrane Discs, Type 146 (con't)</b>	<b>Diameter</b>	<b>10 pc</b>
30 kDa MWCO	25 mm	14659--25-----D
	47 mm	14659--47-----D
	63 mm	14659--63-----D
	76 mm	14659--76-----D
50 kDa MWCO	25 mm	14650--25-----D
	47 mm	14650--47-----D
	76 mm	14650--76-----D
100 kDa MWCO	47 mm	14668--47-----D
	63 mm	14668--63-----D
300 kDa MWCO	25 mm	14679--25-----D
	47 mm	14679--47-----D
	76 mm	14679--76-----D
<b>CTA Membrane Discs, Type 145</b>		
5 kDa MWCO	25 mm	14529--25-----D
	47 mm	14529--47-----D
10 kDa MWCO	25 mm	14539--25-----D
	47 mm	14539--47-----D
	50 mm	14539--50-----D
20 kDa MWCO	25 mm	14549--25-----D
	43 mm	14549--43-----D
	47 mm	14549--47-----D
	63 mm	14549--63-----D
<b>HY Membrane Discs, Type 144</b>		
2 kDa MWCO	47 mm	14419--47-----D
	63 mm	14419--63-----D
5 kDa MWCO	25 mm	14429--25-----D
	44 mm	14429--44-----D
	47 mm	14429--47-----D
	63 mm	14429--63-----D
	76 mm	14429--76-----D
10 kDa MWCO	25 mm	14439--25-----D
	47 mm	14439--47-----D
	63 mm	14439--63-----D
	76 mm	14439--76-----D
30 kDa MWCO	25 mm	14459--25-----D
	47 mm	14459--47-----D
	63 mm	14459--63-----D
	76 mm	14459--76-----D
100 kDa MWCO	47 mm	14468--47-----D



# DNA Concentration

## Table of Contents

Vivacon® 500	46
Vivacon® 2	49

# Vivacon® 500



## 100 to 500 µL samples

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.

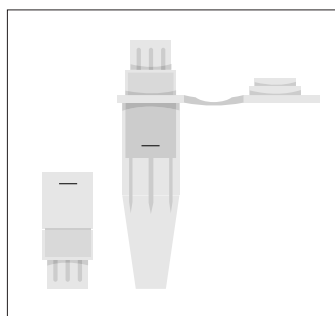
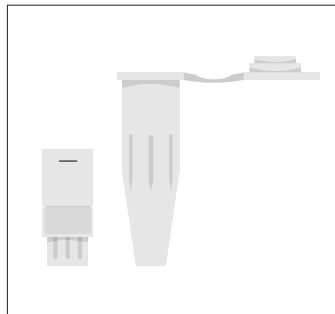
The possibility of a reverse-spin after sample processing assures complete concentrate recovery which is especially important when working with low sample concentrations.

## 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



Reverse spin

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

## Technical Specifications

<b>Concentrator capacity</b>	
Fixed angle rotor	0.5 mL
<b>Dimensions</b>	
Length x diameter	45 x 12.4 mm 47.5 x 12.4 mm reverse spin
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
<b>Materials of construction</b>	
Body	Polycarbonate (PC)
Filtrate vessel	Polypropylene (PP)
Membrane	Hydrosart® (HY) Cellulose Acetate (CA)

## Conversion Table for MWCO to Nucleotide Cut-Off

Membrane	Single-Stranded Cut-Off (SSCO)	Double-Stranded Cut-Off (DSCO)
2 kDa HY	> 10 bases	> 10 bp
10 kDa HY	> 90 bases	> 30 bp
30 kDa HY	> 275 bases	> 50 bp
50 kDa HY	> 475 bases	> 300 bp
100 kDa HY	> 900 bases	> 600 bp
125 kDa CA	> 1,000 bases	> 650 bp

## Typical Performance Characteristics for DNA

Start volume 0.5 mL, sample concentration 50 ng/mL

MWCO	Nucleic Acid Length	Time to Concentrate up to 30× at 20°C	Concentrate Recovery	RCF
2 kDa	10 bp	60 min	93%	7,500 g
10 kDa	30 bp	25 min	94%	7,500 g
30 kDa	50 bp	18 min	88%	5,000 g
50 kDa	300 bp	18 min	91%	5,000 g
100 kDa	600 bp	10 min	87%	3,000 g
125 kDa	650 bp	12 min	85%	2,000 g
125 kDa	900 bp	9 min	94%	3,000 g

## Typical Performance Characteristics for Proteins

Start volume 0.5 mL, sample and concentration of proteins as specified in table

MWCO	Test Molecule	Time to Concentrate up to 30× at 20°C	Concentrate Recovery	RCF
2 kDa	0.25 mg/mL cytochrome c	30 min	95%	14,000 g
10 kDa	0.25 mg/mL cytochrome c	15 min	92%	14,000 g
30 kDa	1.0 mg/mL BSA	10 min	95%	14,000 g
50 kDa	1.0 mg/mL BSA	10 min	92%	14,000 g
100 kDa	1.0 mg/mL bovine IgG	11 min	90%	8,000 g
125 kDa	1.0 mg/mL bovine IgG	10 min	81%	8,000 g

## Ordering Information

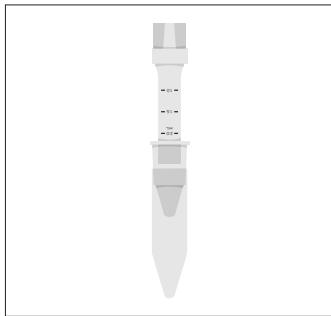
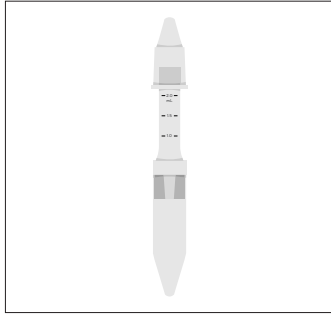
Vivacon® 500	25 pc	100 pc	500 pc
2 kDa MWCO	VN01H91	VN01H92	-
10 kDa MWCO	VN01H01	VN01H02	-
30 kDa MWCO	VN01H21	VN01H22	VN01H23
50 kDa MWCO	VN01H31	VN01H32	VN01H33
100 kDa MWCO	VN01H41	VN01H42	VN01H43
125 kDa MWCO	VN01H81	VN01H82	VN01H83
<b>Vivacon® 500 PCR Grade</b>			
30 kDa MWCO	-	VN01H22ETO	VN01H23ETO
100 kDa MWCO	-	VN01H42ETO	-
125 kDa MWCO	-	VN01H82ETO	VN01H83ETO

## Vivacon® 500 Accessories

Additional collection tubes, 100 pc	VNCT01
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# Vivacon® 2



Reverse spin

## 0.4 – 2 mL samples

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®.

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.

## 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.

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	
Length x diameter	125 x 16 mm 115 x 16 mm reverse spin
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 (PC)
Filtrate vessel	Polypropylene (PP)
Reverse-spin recovery vial	Polypropylene (PP)
Recovery vial cap	Polypropylene (PP)
Membrane	Hydrosart® (HY) Cellulose Acetate (CA)

### Conversion Table for MWCO to Nucleotide Cut-Off

Membrane	Single-Stranded Cut-Off (SSCO)	Double-Stranded Cut-Off (DSCO)
2 kDa HY	> 10 bases	> 10 bp
10 kDa HY	> 90 bases	> 30 bp
30 kDa HY	> 275 bases	> 50 bp
50 kDa HY	> 475 bases	> 300 bp
100 kDa HY	> 900 bases	> 600 bp
125 kDa CA	> 1,000 bases	> 650 bp

### Typical Performance Characteristics for DNA

Volume 2 mL, sample concentration 50 ng/mL, start volume: 2 mL

MWCO	Nucleic Acid Length	Time to Concentrate up to 30× at 20°C	Concentrate Recovery	RCF
2 kDa	10 bp	120 min	92%	7,500 g
10 kDa	30 bp	60 min	94%	5,000 g
30 kDa	50 bp	60 min	95%	2,500 g
50 kDa	300 bp	45 min	96%	2,500 g
100 kDa	600 bp	30 min	93%	2,500 g
125 kDa	650 bp	30 min	88%	2,500 g
125 kDa	900 bp	30 min	89%	2,500 g

### Typical Performance Characteristics for Proteins

Start volume 2 mL, sample and concentration of proteins as specified in table

MWCO	Test Molecule	Time to Concentrate up to 30× at 20°C	Concentrate Recovery	RCF
2 kDa	0.25 mg/mL cytochrome c	120 min	95%	7,500 g
10 kDa	0.25 mg/mL cytochrome c	90 min	96%	5,000 g
30 kDa	1.0 mg/mL BSA	40 min	96%	5,000 g
50 kDa	1.0 mg/mL BSA	30 min	94%	5,000 g
100 kDa	1.0 mg/mL bovine IgG	30 min	92%	5,000 g
125 kDa	1.0 mg/mL bovine IgG	27 min	81%	5,000 g

## Ordering Information

Vivacon® 2	25 pc	100 pc	500 pc
2 kDa MWCO	VN02H91	VN02H92	-
10 kDa MWCO	VN02H01	VN02H02	-
30 kDa MWCO	VN02H21	VN02H22	VN02H23
50 kDa MWCO	VN02H31	VN02H32	-
100 kDa MWCO	VN02H41	VN02H42	VN02H43
125 kDa MWCO	VN02H81	VN02H82	VN02H83
<b>Vivacon® 2 PCR Grade</b>			
30 kDa MWCO	-	VN02H22ETO	-
50 kDa MWCO	-	VN02H32ETO	-
100 kDa MWCO	-	VN02H42ETO	VN02H43ETO
125 kDa MWCO	-	-	VN02H83ETO



# Protein Purification

## Table of Contents

Vivaclear Centrifugal Filters	54
Vivapure® Ion Exchange Purification Products	56

## Vivaclear Centrifugal Filters



Vivaclear centrifugal filters are disposable microfiltration devices for the fast and reliable clarification or filtration of biological samples in the range 100 to 500  $\mu\text{L}$ . They can be used in fixed angle rotors accepting 2.2 mL centrifuge tubes.

### Applications

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

### Product Features

- High-flux polyethersulfone membrane
- 0.8  $\mu\text{m}$  pore size
- Low hold-up volume (<5  $\mu\text{L}$ )
- Fast and reproducible performance

## Technical Specifications

<b>Filter capacity</b>	
Swing bucket rotor	do not use
Fixed angle rotor	500 $\mu$ L
<b>Dimensions</b>	
Length x diameter	43 x 11 mm
Active membrane area	0.34 cm <sup>2</sup>
Hold-up volume, membrane and support	< 5 $\mu$ L
<b>Materials of construction</b>	
Body	Polypropylene (PP)
Filtrate tube	Polypropylene (PP)
Membrane	Polyethersulfone (PES)

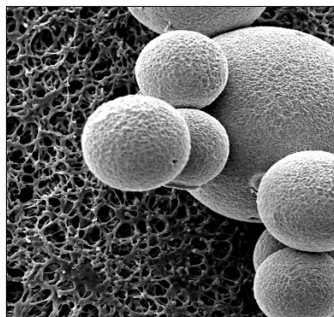
## Equipment Required

<b>Centrifuge</b>	
Rotor type	Fixed angle (40-45°)
Rotor cavity	To fit 2.2 mL (11 mm) conical bottom tubes
Maximum RCF	2,000 <i>g</i>
<b>Filtrate recovery</b>	
Pipette type	Fixed or variable volume
Recommended tip	Standard type

## Ordering Information

Vivaclear Mini	100 pc
0.8 $\mu$ m PES	VK01P042

# Vivapure® Ion Exchange Purification Products



SEM comparing chromatography beads (right) with the Sartobind® membrane adsorber, which features 50x larger pore sizes.

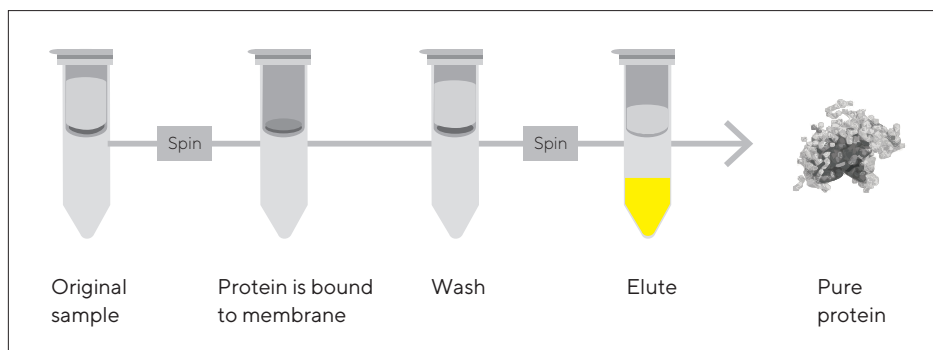
## Fast and easy-to-use spin columns

Vivapure® Ion Exchange (IEX) devices incorporate Sartobind® membrane adsorber technology as the chromatography matrix. The ready-to-use spin column format makes protein purification as easy as filtration. With no risk of running dry, Vivapure® replaces time-consuming and expensive resin-based chromatography in many protein purification workflows.

The rapid bind-wash-elute protocol is especially ideal in screening applications, where multiple samples or purification conditions can be conveniently processed in parallel.

The microporous structure of these membrane adsorbers has a pore size  $> 3 \mu\text{m}$ , which is orders of magnitude larger than conventional chromatography resins. This allows molecules to be transported to the ligands immobilized on the membrane adsorber by convective flow, overcoming the diffusion limitations of chromatography resins, and leading to very high flow rates. The large pore sizes also prevent gel filtration effects and minimize non-specific binding.

With Vivapure®, there is no need for column packing, saving time and ensuring reproducibility. Furthermore, Sartobind® membrane adsorber technology is available in process scale formats, making Vivapure® an indispensable tool for process development prior to purification scale-up.



Fast and easy protein purification with Vivapure® spin columns

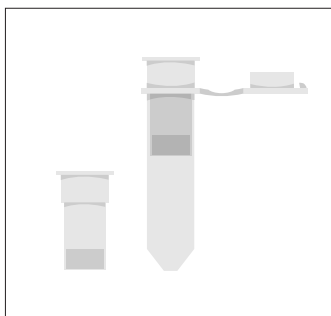




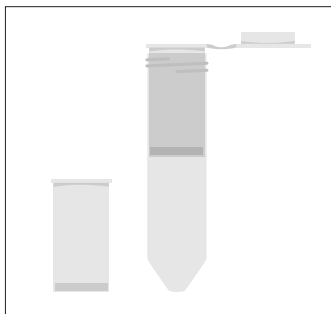
Vivapure® Mini H



Vivapure® Maxi H



Vivapure® Mini H



Vivapure® Maxi H

## Membrane Availability

Functional groups	Ion exchanger type
Sulphonic acid (S)	Strong acidic cation exchanger: R-CH <sub>2</sub> -SO <sub>3</sub> <sup>-</sup> Na <sup>+</sup>
Quaternary ammonium (Q)	Strong basic anion exchanger: R-CH <sub>2</sub> -N <sup>+</sup> -(CH <sub>3</sub> ) <sub>3</sub> Cl <sup>-</sup>
Diethylamine (D)	Weak basic anion exchanger: R-CH <sub>2</sub> -NH <sup>+</sup> -(CH <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>

## Typical Performance Characteristics

Vivapure® spin columns	Protein binding capacity*	Max. volume, swing bucket	Max. volume, fixed angle
Vivapure® Mini H	4 mg	-	0.4 mL
Vivapure® Maxi H	60 – 80 mg	19 mL	10.5 mL

## Typical Applications

- Fractionation of protein mixtures prior to 1D or 2D-PAGE
- Scouting purification conditions for new protein targets
- Removal of endotoxins from monoclonal antibodies
- Preparation of heme moiety from heme containing protein prior to functional analysis
- General protein purification and polishing
- Detergent removal from protein solutions
- Purification of antibodies from serum, ascites or cell culture supernatant
- Intermediate sample purification prior to further HPLC | FPLC
- Purification of membrane-bound proteins

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

## Ordering Information

Vivapure® Mini	Spin Columns	Centrifuge Tubes	Prod. No.
Vivapure® D Mini H	24	48	VS-IX01DH24
Vivapure® Q Mini H	24	48	VS-IX01QH24
Vivapure® S Mini H	24	48	VS-IX01SH24
<b>Vivapure® Maxi</b>			
Vivapure® D Maxi H	8	16	VS-IX20DH08
Vivapure® Q Maxi H	8	16	VS-IX20QH08
Vivapure® S Maxi H	8	16	VS-IX20SH08

\* Binding capacities established using 1 mg/mL BSA in 25 mM Tris-HCl (pH 8) for Vivapure® Q and D devices, or 1 mg/mL cytochrome c in 25 mM sodium acetate buffer (pH 5.5) for Vivapure® S devices. Actual capacities depend on the target molecule and selected buffer conditions.



Technico

10

SARTOBIND® Q15 Lot no. 815

# Virus Purification and Concentration

## Table of Contents

Vivapure® Virus Purification and Concentration Kits	60
Adenovirus Purification	61
Vivapure® Adenopack 20	62
Vivapure® Adenopack 100	63
Vivapure® Adenopack 500	65
Lentivirus Purification	66
Vivapure® Lentiselect 40	67
Vivapure® Lentiselect 500	68
Vivapure® Lentiselect 1000	69

# 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

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

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

## 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 invitro 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.

## Adenopack Advantages

### Fast and Easy Virus Purification

- Purification completed in 2 hours
- Convenient, over 10 × 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 of GFP-Ad5 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%	$1 \times 10^{11-12}$

\* 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.com](http://www.sartorius.com). Additionally, the tubes and adaptors needed for these operation modes can be ordered.

\*\* 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.

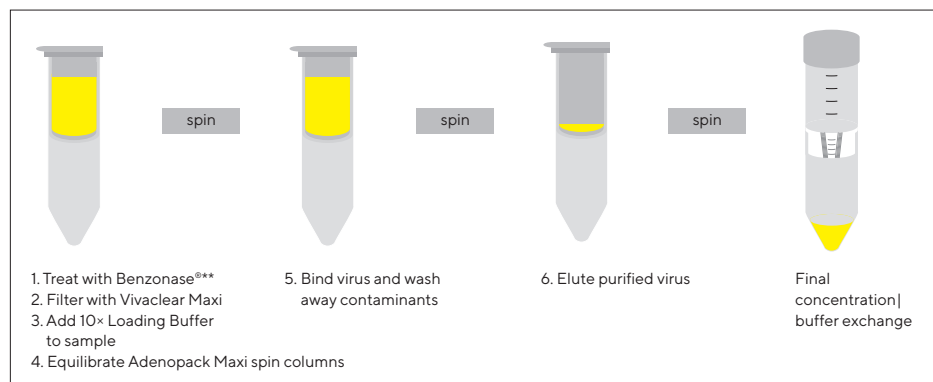
## Ordering Information and Kit Contents

Vivapure® Adenopack 20	VS-AVPQ020
Vivapure® Adenopack 20 RT*	VS-AVPQ022
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



\* Adenopack 20 RT does not include Benzonase®

\*\* 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.

## Ordering Information and Kit Contents

Vivapure® Adenopack 100	VS-AVPQ101
Vivapure® Adenopack 100 RT*	VS-AVPQ102
Adenopack 100 units	2
Minisart® NML 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

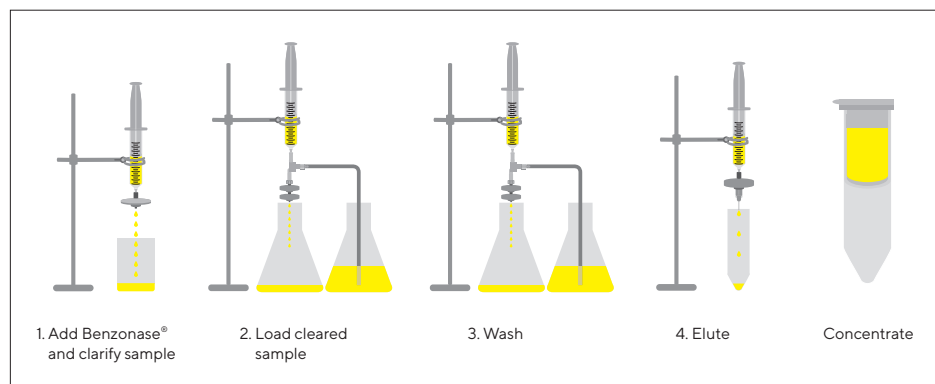
Pump tubing set for Vivapure® Adenopack 100	VS-AVPA001
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\* Adenopack 100 RT does not include Benzonase®

## 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,  $25 \times 15$  cm culture plate purified using this method yields up to  $3 \times 10^{13}$  viral particles.

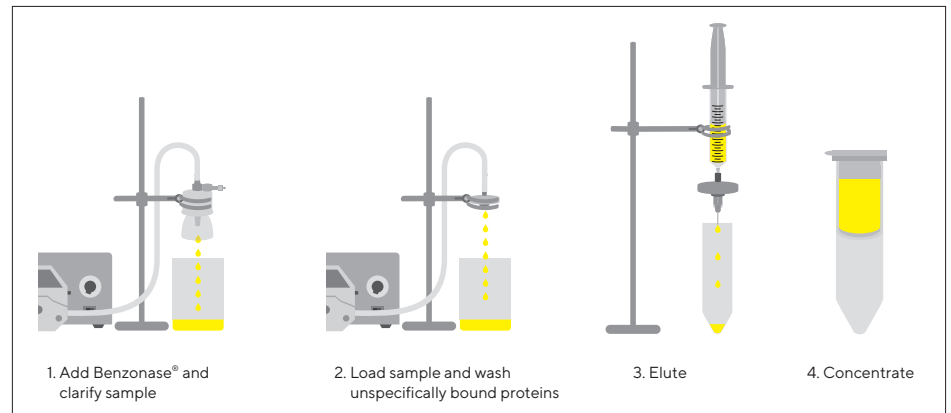
## Ordering Information and Kit Contents

Vivapure® Adenopack 500	VS-AVPQ501
Vivapure® Adenopack 500 RT*	VS-AVPQ502
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



\* Adenopack 500 RT does not include Benzonase®

# Lentivirus Purification

## 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 1 L 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 of 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 1 L 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<sup>®</sup> Lentiselect 40

Fast purification of up to  $8 \times 10^8$  viral particles



Vivapure<sup>®</sup> 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<sup>®</sup> Lentiselect is fast and simple, without the need for expensive equipment like an ultra-

centrifuge. Additionally, 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.

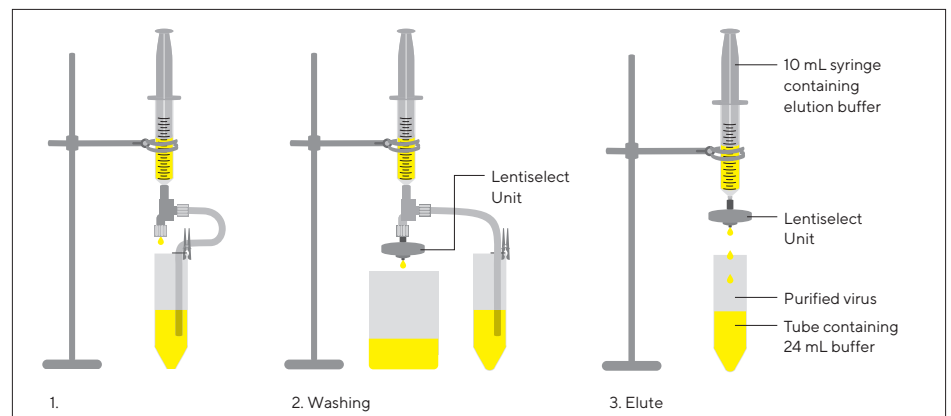
## Ordering Information and Kit Contents

Vivapure <sup>®</sup> Lentiselect 40	VS-LVPQ040
Lentiselect unit	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 <sup>®</sup> 20, 100 kDa MWCO	8
Instructions	1 each for Kit and Vivaspin <sup>®</sup>

## Technical Data

### Kit specifications

Sample size	40 mL cell culture
Number of purifications	4 × 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 ultracentrifugation,

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 per millilitre (total volume 1 mL).

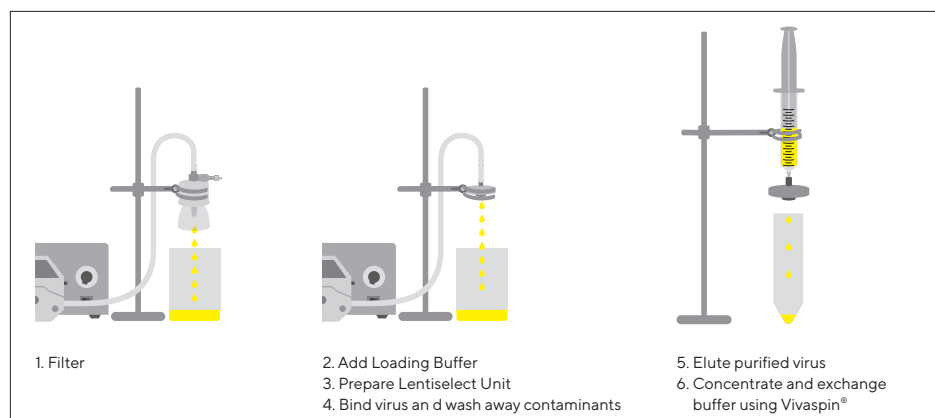
## Ordering Information and Kit Contents

Vivapure® Lentiselect 500	VS-LVPQ500
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, 100 kDa MWCO	2
Operating manual	1 each for Kit and Vivaspin®

## Technical Data

### Kit Specifications

Sample volume	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 1 L cell culture supernatant.

This kit is to be operated by a laboratory pump and contains all necessary buffers and ultrafiltration

devices for optimal convenience. The traditional time consuming ultracentrifugation method is replaced by this fast and simple Vivapure® Lentiselect 1000 kit.

## Typical Performance

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

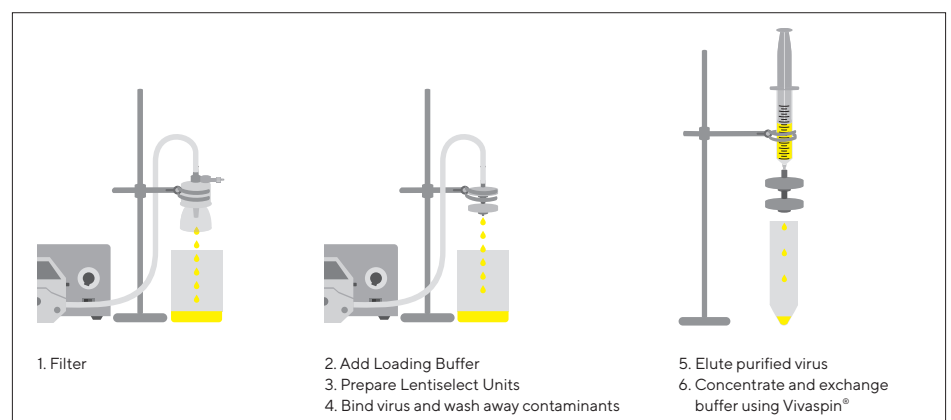
## Ordering Information and Kit Contents

Vivapure® Lentiselect 1000	VS-LVPQ1000
Lentiselect unit	2
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	60 mL
Vivaspin® 20, 100 kDa MWCO	2
Operating manual	1 each for Kit and Vivaspin®

## Technical Data

### Kit specifications

Sample volume	1 L cell culture
Number of purifications	1 × 1 L
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

## Table of Contents

1. Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators	72
2. Treatment of Vivaspin® Concentrators for Improved Recovery of Low-concentrated Protein Samples	75
3. Scouting Protein Purification Conditions Using Vivapure® Centrifugal Ion Exchange Membrane Absorbers	78
4. Concentration and Purification of Viruses by using Ultrafiltration, Incl. Coronavirus – a Short Review	83
5. Sartorius Ultrafiltration Products in the Preparation of Biological Nanoparticles and Medical Nanocarriers	95
6. Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories	101

# 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 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 100-fold still contains 500 mM salt. If this concentrate is then diluted 100× with water or salt-free buffer, the protein concentration returns to the original level, while the salt concentration is reduced 100× to only 5 mM (i.e. a 99% reduction in salt concentration).

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 10×. For example, if a precipitous protein sample is concentrated to 5× 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.



## Methods

Select an appropriate MWCO for your sample. For maximum recovery, select a MWCO  $\frac{1}{3}$  to  $\frac{1}{2}$  the molecular weight of the molecule of interest.

1. Add protein sample up to the maximum fill volume of the concentrator (as stated in the device operating instructions). If the sample volume is lower than the maximum device volume, it can be diluted to the maximum fill volume before the first centrifugation step. This will increase the salt removal rate.
2. Centrifuge for the recommended amount of time at an appropriate spin speed (see device operating instructions).
3. Empty filtrate container and refill the concentrator with an appropriate exchange solvent.\*
4. Centrifuge again as before.
5. Recover the concentrated, desalted sample from the bottom of the concentrate pocket with a pipette.

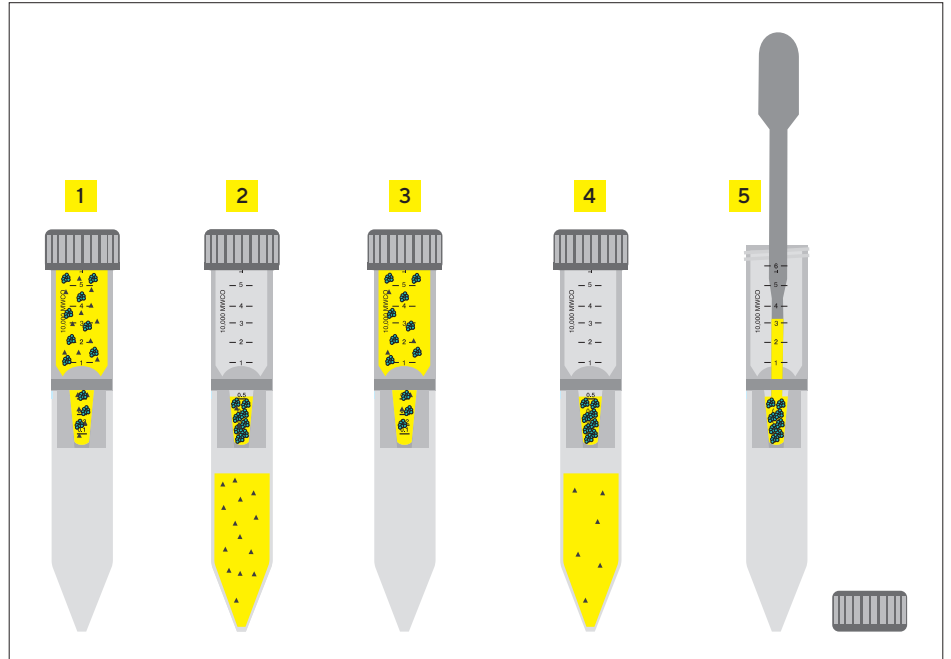


Figure 1: Step-by-step method for desalting and concentration

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

## Results

### 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 MWCO were tested with 20 mL samples. Each sample contained 500 mM NaCl. To perform diafiltration, devices were centrifuged at 4,000 g for 45 min (5 kDa MWCO) or 30 min (>5 kDa MWCOs). After the first and second spins, the retentate samples were

brought up to 20 mL with ultrapure water from an Arium® system (Sartorius). OD readings were taken at 410 nm for Cytochrome C or 280 nm for BSA and IgG samples. Salt concentrations were measured using 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 MWCO were tested with 6 mL samples. Each sample contained 500 mM NaCl. To perform diafiltration, devices were centrifuged at 4,000 g for 45 min (5 kDa MWCO) or 30 min (>5 kDa MWCOs). After the first and second spins, the retentate samples

were brought up to 6 mL with ultrapure water from an Arium® system (Sartorius). OD readings were taken at 410 nm for Cytochrome C or 280 nm for BSA and IgG samples. Salt concentrations were measured using a Qcond 2200 conductivity measuring instrument.

### Conclusions

As the results 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 target protein. Diafiltration using ultrafiltration devices such as Vivaspin® 6 and 20 represents a faster and more efficient solution to desalting and buffer exchange, than conventional techniques such as dialysis.

## 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 – 4 × 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 (Figure 1) 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 (Figure 2) 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.

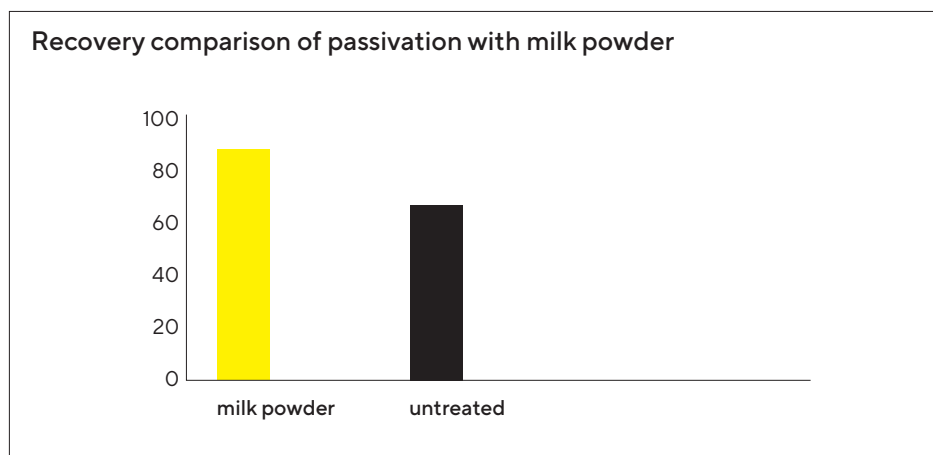


Figure 1: Protein recovery (10 µg/mL BSA) with Vivaspin® PES 10 kDa after passivation

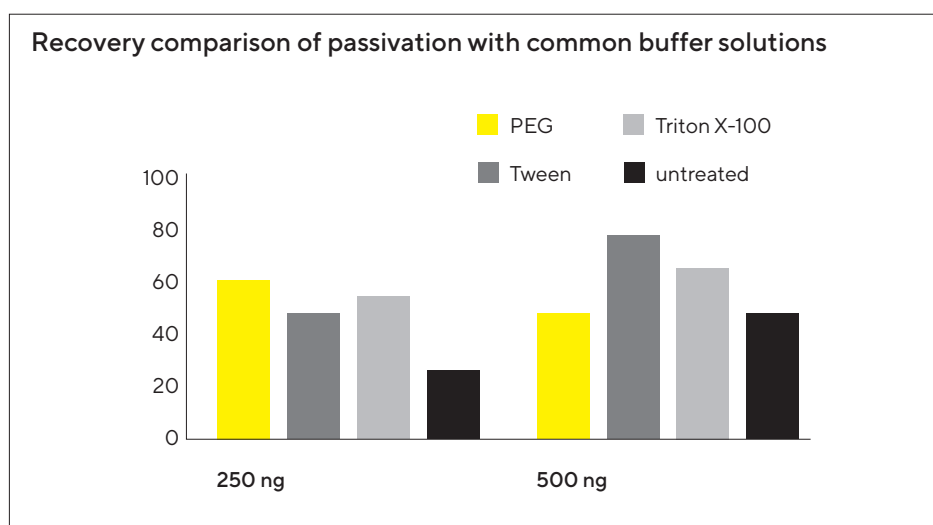


Figure 2: Protein recovery (250 and 500 ng BSA) with Vivaspin® 2 PES 10 kDa after passivation

### 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 recommended 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.

### 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 charge differences between biomolecules. This makes it 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 identifying a purification method based on ion exchange chromatography for a given target protein, as it is fast and only uses small amounts of the sample.

In the first step of this study, binding conditions were 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.

The results from this experiment provided the optimal binding pH and the best ion exchange chemistry for the purification of SH2 domain.

In a second step, the best elution method was 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.

#### Materials and Methods

Buffers tested to determine the optimum pH and salt concentration for binding and elution in ion exchange purification of SH2 domain

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Buffer A:	25 mM Citrate, pH 4
Buffer B:	25 mM Potassium phosphate, pH 6
Buffer C:	25 mM HEPES, pH 8
Buffer D:	25 mM Sodium bicarbonate, pH 10
Buffer E:	25 mM Citrate, pH 4, supplemented with 1 M NaCl.
Buffer F:	25 mM Potassium phosphate, pH 6, supplemented with 0.2 M, 0.4 mM, 0.6 mM, 0.8 mM and 1 M NaCl, respectively.
Buffer G:	25 mM HEPES, pH 8, supplemented with 1 M NaCl
Buffer H:	25 mM Sodium bicarbonate, pH 10, supplemented with 1 M NaCl

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### Scouting Binding Conditions

300 mL LB media was inoculated with 4 mL of an overnight culture and incubated at 37°C, shaking at 150 rpm until an OD600 of 1.0 was reached. IPTG was added to a final concentration of 1 mM and the culture incubated for a further 4 h with shaking at 150 rpm. Cells were harvested by centrifugation at 4,000 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 and cell debris were removed by centrifugation at 10,000 g for 30 min at 4°C.

4 × 200 µL aliquots of the cell lysate were diluted with 1.8 mL binding buffer A to D, to adjust each sample to the respective pH being tested. To avoid clogging of the membranes in the Vivapure® Mini spin columns, samples were clarified by passage through 0.45 µm CA Minisart NML syringe filters (Sartorius).

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 was added and spun for 5 minutes at 2,000 g (45° fixed angle rotor).

400 µL of the clarified samples adjusted to pH 4, 6, 8 or 10 were applied to each of the correspondingly equilibrated Vivapure® Q and S spin columns. Columns were spun for 5 min at 2,000 g.

Afterwards, Vivapure® Mini spin columns were reloaded with 400 µL sample and spun again for 5 min at 2,000 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 2,000 g. Flow-through and wash fractions were collected for subsequent detection of the target protein.

200 µL of elution buffer E, F, G or H, were applied to the washed columns and spun for 3 min at 2,000 g. Eluates were saved for subsequent analysis.

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

### Optimizing Elution Conditions

Taking account of the results of the first experiment (Scouting Binding Conditions) 200 µL cell lysate was diluted with 1.8 mL binding buffer B (25 mM KPi, pH 6). To avoid clogging of the membrane in the Vivapure® Mini spin column, the pH adjusted sample was clarified by passage through a 0.45 µm CA Minisart NML syringe filter (Sartorius).

400 µL binding buffer B was applied to one Vivapure® S Mini spin column and spun for 5 minutes at 2,000 g.

400 µL of the clarified sample was applied to the equilibrated Vivapure® S column and spun for 5 min at 2,000 g. Afterwards, the Vivapure® S Mini spin column was reloaded with 400 µL sample and spun again for 5 min at 2,000 g.

Loosely bound proteins were washed away by application of 400  $\mu$ L binding buffer to the column and spinning for 5 min at 2,000 g. Flow-through and wash fractions were saved for analysis.

To elute the target protein, 100  $\mu$ L elution buffer F, supplemented with 0.2 M NaCl was applied to the Vivapure<sup>®</sup> S Mini spin column and spun for 3 min at 2,000 g. The eluate was collected. For the next elution step, 100  $\mu$ L of elution buffer F, supplemented with 0.4 M NaCl was applied and again spun for 3 min at 2,000 g. Elution was continued with 0.2 M NaCl increments until a final salt concentration of 1 M was reached, saving the eluates from each step.

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

## Results

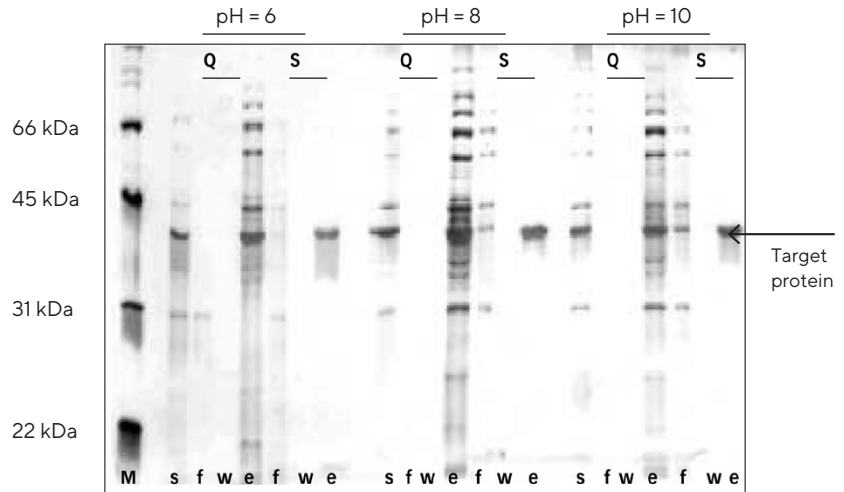
Dilution of the *E. coli* lysate with binding buffer A (25 mM Citrate, pH 4) lead to complete precipitation of sample proteins. Thus, pH 4 could not be tested in this experiment. As can be seen on the SDS gel in (Figure 1), the target protein was present in the eluates from Vivapure<sup>®</sup> Q Mini spin columns 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 S “f”), thus resulting in purer target protein in all eluate fractions (Lane S “e”).

Differences could be detected in the binding efficiency of the target protein. 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.

The purification conditions determined for Vivapure<sup>®</sup> S with potassium phosphate buffer (pH 6) were further optimized to determine the ideal salt concentration for SH2 domain elution. 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.



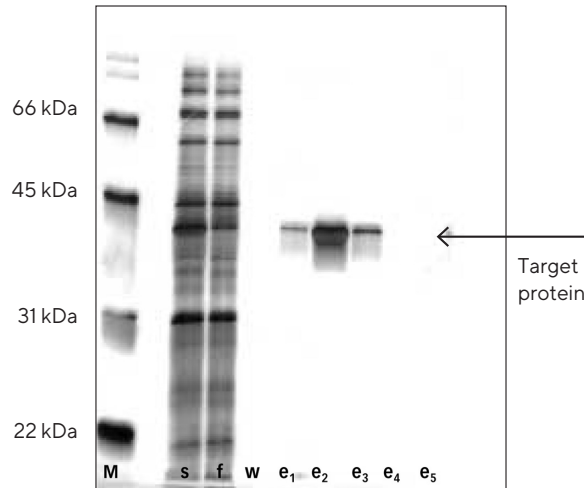
Figure 1



Sample	Sample Volume ( $\mu\text{L}$ )	Volume Loaded Onto Gel ( $\mu\text{L}$ )
M = Broad range marker		
s = Sample before application	800	4
f = Flow-through	800	4
w = Wash fraction	400	4
e = Elution with 1 M NaCl	200	4

**Note:** Scouting for optimal binding conditions of a SH2 domain expressed in *E. coli*. 12% reducing SDS gel, silver stained, shows the sample before purification (s), flow-through (f), wash (w) and eluate (e) fractions (1 M NaCl) from Vivapure® Q and S Mini spin columns, at the various pH values tested.

**Figure 2**



Sample	Process Volume (μL)	Volume Loaded Onto Gel (μL)
M = Broad range marker		
s = Sample before application	800	16
f = Flow-through	800	16
w = Wash fraction	400	16
e1 = 25 mM KPi, pH 6, 200 mM NaCl	100	8
e2 = 25 mM KPi, pH 6, 400 mM NaCl	100	8
e3 = 25 mM KPi, pH 6, 600 mM NaCl	100	8
e4 = 25 mM KPi, pH 6, 800 mM NaCl	100	8
e5 = 25 mM KPi, pH 6, 1 M NaCl	100	8

**Note:** Optimizing elution conditions for a SH2 domain expressed in *E. coli*, using Vivapure® S Mini spin column at pH 6. 12% reducing SDS gel, silver stained, shows the sample before purification (s), flow through (f), wash (w) and eluate (e1-5) fractions.

### Conclusion

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 suitable ion exchanger and buffer pH for binding the target protein was verified. In the second step, the elution condition was optimized, building on the results gained in step one. 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 to various ends, e.g:

- Polishing a specific protein after purification with another chromatographic technique
- Quickly establishing a FPLC method for a new protein
- Identification of the optimal purification method prior to scale up with Vivapure® IEX Maxi spin columns.

For these purposes Vivapure® IEX Mini and Maxi spin columns and Sartobind® membrane adsorber units with FPLC connectors are available.

## 4. Concentration and Purification of Viruses by Using Ultrafiltration, Incl. Coronavirus – A Short Review

### Introduction

Evolutionary, viruses developed various mechanisms to interact and manipulate the genetic material of their target cells. Based on this, modern molecular biology utilizes viruses in a constantly growing number of applications.<sup>1</sup> They range from controlled genetic transfection of cells to a variety of different basic studies in medical science.<sup>2</sup> In medical studies the strategic focus is on recombinant vaccines and on the development of potential vectors for gene therapy.<sup>3,4</sup>

Besides the great relevance of viruses for medical applications, the assessment of virus type and content is important for the risk assessment of food and drinking water.<sup>5</sup> Also, the classification of virus content is often of high relevance for the quality control of aquatic biotopes.<sup>6</sup>

During the preparation, handling, or analysis of viruses or virus-like particles (VLPs), a concentration and/or purification step is frequently required.<sup>5</sup> Typical viruses have a size within the range of about 20 nm up to several hundred nanometers.<sup>7</sup> Therefore they are ideally suited for the retention on ultrafiltration membrane systems and such ultrafilters are widely used in basic virus research. The specifications of such ultrafiltration devices depend on the particular type of virus and the purpose of the subsequent application.

This short review highlights methods for the purification of various mammalian viruses for basic medical research. Also, the concentration of pathogenic viruses from water and food samples and the purification of marine bacteriophages (virioplankton) are highlighted. It will also give guidance for the selection of an ideal performing device with the optimum molecular weight cut-off (MWCO) for the user specified ultrafiltration process.

### Concentration of mammalian viruses in medical research

In medical research viruses and VLPs are of major interest, particularly for investigations on infectious viral diseases and for the development of vaccines or antiviral drugs. Moreover, certain VLPs can manipulate genetic material in a directed manner and are used broadly in the development of genetic therapy approaches. Additionally, viral vectors are well established as a transfection method for gene transfer to cell lines e. g. to manipulate mammalian cells in vivo and in vitro.

An overview of thematically linked publications using Sartorius ultrafiltration devices for the purification and concentration of viruses and VLPs in the medical context is given in Table 1. Among other applications, Vivaspin® devices were employed for the concentration of adeno-associated virus (AAV) and lentiviral vectors after purification via ion exchange chromatography,<sup>8-10</sup> on blood sera to prepare blank samples from hepatitis C virus (HCV)-positive blood sera,<sup>11</sup> for the development of a vaccine against human immunodeficiency virus (HIV) and of an antiviral drug against Chikungunya virus.<sup>12,13</sup>

**Table 1: Summarized examples of applications using Vivaspin® and Vivaflow® for viruses in medical research**

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Gene therapy (Adenovirus type 5, VLP, human)	Diafiltration (20 mM Tris saline buffer)	Vivaflow® (100 kDa)	Storage, chromatography on Sartobind® STIC membrane absorber (FPLC)	14
Reduction of HCV-induced fibrosis (Hepatitis C Virus; human)	Removal of HCV from human blood serum (Blood serum)	Vivaspin® (30 kDa)	Preparation of negative control (from positive sample) for immunofluores- cence assay, fibrosis induction assays	11
Development of a viral entry inhibitor for HIV (HIV, human)	Removal of protein fraction from virus (PBS)	Vivaspin® 20 (1,000 kDa)	Virus inactivation	12
Gene therapy for cancer treatment (adeno-associated virus; rAAV-2, human)	Concentration and purification after expression, Buffer exchange after His tag (FreeStyle 293 Expression Medium (Gibco), serum-free)	Vivaspin® 20 (1,000 kDa)	Titer, ELISA, cell binding assay, apoptosis   cell cycle assay	8
System for controlled gene expression in mice brain (Adeno- associated virus, mice)	Concentration of eluate after anion exchange chromatog- raphy (elution buffer)	Vivaspin® 20 (100 kDa)	Transduction of mice neurons	9
Efficient gene transfer into the CNS (Lentivirus, human)	Concentration after ion exchange chromatography (PBS)	Vivaspin® (100 kDa)	Quantification via real- time PCR and end-point dilution. Transduction of murine neuronal and glial cells in vivo	10
Identification of effective chikungunya antiviral drugs (Chikungunya-Virus, human)	Concentration	Vivaspin® 20 (100 kDa)	Quantification by TCID <sub>50</sub>	13
Gene therapy of achromatopsia in mice (Recombinant adeno-associated virus, human virus used in mice)	Concentration (Anion exchange chromatography elution buffer)	Vivaspin® 4 (10 kDa)	Titer determination by dot-blot analysis, subretinal injections	15

### Concentration of viruses from drinking water and food samples

The guidelines for drinking-water quality by the world health organization describe safety plans to reduce potential risks from various virus infections.<sup>16</sup> It states that, due to the increased resistance of viruses to disinfection methods, an absence of bacterial contamination after disinfection cannot be used as a reliable indicator of the presence | absence of pathogenic viral species in drinking water supplies. Considering this, ultrafiltration can play a vital role in detecting such viral contaminations for the research on drinking water quality and food safety.

For an ultrafiltration step, the water sample does not have to be pre-conditioned and its efficacy in concentrating the virus is virtually

independent of the chemical properties and the structure of the virus.<sup>17</sup> Thus ultrafiltration is very well suited to isolate and concentrate virus particles from water samples and is a valuable aid during the assessment of water quality. Most of the viruses which are found in water and also food samples are of fecal origin. Screening for these viruses is crucial to prevent infections. The most frequent ones are hepatitis A, hepatitis E and norovirus.<sup>18</sup> Ultrafiltration has been described as the most appropriate method for the recovery of hepatitis A virus from vegetables and other food items.<sup>19</sup> Detection of infectious viruses is mainly done by propagation in cell culture (plaque assay) or the detection of the viral genomes by molecular amplification techniques such as quantitative reverse transcriptase polymerase chain reaction (RT-PCR).<sup>20</sup>

**Table 2: Summarized examples of ultrafiltration applications using Vivaspin® and Vivaflow® for viruses from drinking water and food samples**

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultra-filtration Device (MWCO)	Subsequent Step	Ref.
Method for the detection of norovirus genogroup I (Norovirus, human)	Concentration (PBS processed food samples)	Vivaspin® (5 kDa)	RNA extraction for real-time RT-PCR	22
Analysis of viral content in groundwater (A set of pathogenic viruses, potentially human)	Concentration of drinking water sample (Drinking water)	Vivaflow® 200 (10 kDa)	Qualitative analysis (enterovirus) by RT-nested PCR and microtiter neutralization test	21
Comparative Analysis of Viral Concentration Methods (Hepatitis A virus, human)	Concentration (0.25 M threonine, 0.3 M NaCl, pH 9.5)	Vivaspin® 20 (100 kDa)	RNA extraction for real-time RT-PCR	19
Analysis of regional outbreak of gastro-enteritis due to drinking water contamination (Norovirus, Astrovirus, Rotavirus, Enterovirus, Hepatitis A virus; human)	Concentration (50 mmol/L glycine buffer, 1% beef extract)	Vivaspin® 2	Nucleic acid extraction	23

### Concentration of viruses and bacteriophages from marine biological samples

In marine biology, the concentration and subsequent analysis of marine bacteriophages (virioplankton) is of major interest. They outnumber the bacterioplankton (their host organisms) by an order of magnitude and thus have an important influence on the whole marine biosphere.<sup>24</sup>

As described by Wyn-Jones & Sellwood (ref. 17) ultrafiltration can be used to concentrate virus particles in water samples without any prior pretreatment of the sample and it is also practically independent from the

chemical and structural properties of the viruses. Thus, it finds wide use for the analysis of aquatic viruses. For instance, Schroeder et al. (ref. 26) were able to determine the diversity and monitor population dynamics of viruses that infect *Emiliana huxleyi*, a globally important form of photosynthetic plankton. In this study a reusable Vivaflow® 50 unit equipped with a polyethersulfone (PES) membrane with MWCO of 50 kDa was used to concentrate viruses in sea water samples prior to storage and analysis. For further examples of virus concentration from marine biological samples see table 3.

**Table 3: Summarized examples of ultrafiltration applications using Sartorius Vivaflow® and Vivaspin® for samples from marine biology**

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultra-filtration Device (MWCO)	Subsequent Step	Ref.
Assessment of viroplankton diversity (Viroplankton, Plankton)	0.2 µm filtration for clarification, filtrate subjected to 3 kDa filter for concentration (Sea water)	Vivaflow® 200 (0.2 µm and 30 kDa)	Subsequent analysis by DNA separation on Agarose gel	25
Classification of virus (MpRNAV-01B, <i>Micromonas pusilla</i> )	Vivaflow® 200: harvest and concentration of whole cell lysate; Vivaspin®: washing (removal of CsCl)	Vivaflow® 200, Vivaspin® (30 kDa)	Classification of new virus: genome, proteins, stability, etc.	28
Assessment of genetic diversity in viroplankton ( <i>Emiliana huxleyi</i> Bloom virus, Eukaryotic phytoplankton - alga)	After 0.45 µm filtration, concentration 1 L to 20 mL (Sea water)	Vivaflow® 50 (50 kDa)	PCR and Denaturing gradient gel electrophoresis	26
Investigation of gene expression during infection ( <i>Emiliana huxleyi</i> virus strain 86, Eukaryotic phytoplankton - alga)	Concentration from 5 L to 20 mL (f/2 medium)	Vivaflow® 50 (50 kDa)	CsCl-gradient	27
Study on host genome integration ( <i>virophage mavirus</i> , <i>Cafeteria roenbergensis</i> )	Clarification with 0.2 µm filter and concentration with 100 kDa filter ( <i>Cafeteria roenbergensis</i> , f/2 medium)	Vivaflow® 200 (0.2 µm and 100 kDa)	CsCl gradients, electron microscopy	29

### **Concentration of Coronavirus for general research and protein research (spike protein)**

Coronaviruses are spherical, enveloped, RNA based viruses that are typically 80–120 nm in diameter, but in many cases have a diameter outside of this range. Coronavirus genomes are the largest of all RNA viruses which offers a relatively large area of study. Correlatingly the potential for future mutations in this large genome may lead to future human diseases that may evolve into epidemics and pandemics, such as the previous Middle East Respiratory Syndrome (MERS-CoV), and Severe Acute Respiratory Syndrome 1 (SARS-CoV-1) and 2 (SARS-CoV-2). Hence further research into the replication, transmission, genome and structure will continue with greater investment of time and funding in the years to come.

A key component to the infection cycle is the coronavirus spike (S) protein, that mediates entry into host cells, through both attachment and membrane fusion. As such, it is a primary target for the development of novel antiviral drugs and vaccines.

The concentration and purification of both the virions and the spike proteins from cell culture and supernatants is often a key requirement to isolate the respective target, prior to structural, functional analysis and binding assays, etc.

Table 4 highlights several applications where Vivaspin® centrifugal concentrators, or Vivaflow® tangential flow filtration cassettes have been used for the concentration or Coronavirus proteins, including the spike protein. References are also provided to direct readers for detailed reading.

Table 5 provides examples of concentration of intact virions, or Coronavirus virus like particles (VLPs), with the same devices.

**Table 4: Summarized examples of ultrafiltration applications using Sartorius Vivaflow® and Vivaspin® for coronavirus protein samples.**

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Neutralization of a SARS-CoV-2 antibody to a functionally conserved receptor binding domain (RBD) on the trimeric spike (S) protein	Buffer exchange of a SARS-CoV-2 RBD protein	Vivaspin® 20, PES (10 kDa)	Protein concentration by UV/Vis and binding affinity by Streptavidin BLI (Sartorius Octet)	30
Investigation of neutralising antibody response on a SARS-CoV-2 spike glycoprotein RBD-SpyVLP (virus-like particle) platform	Concentration of SpyTag-RBD protein construct	Vivaspin® 20, PES (10 kDa)	Purification by SEC	31
Investigation of exosome based vaccines containing coronavirus spike (S) protein, for SARS-CoV-1	Concentration of solubilized spike protein in supernatant	Vivaspin®, PES (10 kDa)	Western blot analysis	32
Analyze of the ability to redirect the functionality of the Mouse Hepatitis coronavirus spike (S) protein to infect human cancer cells	Concentration of cellular receptor protein constructs	Vivaspin®, PES	Western blot analysis	33
Structure determination of coronavirus SARS-CoV-1 non-structural protein 1 (nsp1)	Concentration of coronavirus nsp1 during purification process	Vivaspin®, PES	Crystalization screening	34
Structure determination of the ADRP domain of Feline Coronavirus (FCoV) non-structural protein 3 (nsp3)	Concentration of coronavirus nsp3 during purification process	Vivaspin®, PES (10 kDa)	Crystalization screening	35
Investigation into the role of three transmembrane proteases in the activation of SARS-CoV-1 spike (S) protein	Concentration of VLPs from HEK 293T cell culture supernatant	Vivaspin®, PES	Cell-cell fusion assay	36
Cryo-electron microscopy of Human Coronavirus HCoV-NL63 spike glycoprotein trimer that is a potential target for neutralizing antibodies during infection	Concentration of recombinant HCoV-NL63 viruses from clarified Drosophila S2 cell culture supernatant	Vivaflow®, PES (10 kDa)	Affinity purification	37



**Table 5: Summarized examples of ultrafiltration applications using Sartorius Vivaspin® for coronavirus viron and VLP samples**

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Characterisation of phenotypic changes in virus isolates, such as MERS-CoV, that could relate to pandemic potential	Concentration of MERS-CoV virus isolates	Vivaspin®, PES (100 kDa)	Quantification using plaque titration Viral RNA sequencing analysis	38
Investigation of antiviral potential of Echinacea purpurea (Echinaforce®) against human coronaviruses; SARS-CoV and MERS-CoV	Concentration of MERS-CoV and SARS-CoV virus dilutions	Vivaspin® 20, PES	Limiting dilution assay (TCID <sub>50</sub> )	39
Investigation into inactivation of SARS-CoV-2 through heating and chemical protocols	Concentration and separation of deactivated SARS-CoV-2 from lysis buffer	Vivaspin® 500, PES	Inoculation onto Vero-E6 monolayer	40
Investigation of viral and cellular determinants governing hCoV-EMC entry into host cells	Concentration of SARS-CoV and hCoV-EMC virus like particles (VLPs)	Vivaspin®, PES	Western blot analysis	41, 42

### **Concentration and capture of virions and | or viral RNA in wastewater**

In humans and birds Coronaviruses may inflict mild to fatal respiratory tract infections, but in other animal groups a range of other diseases may also occur, such as hepatitis and neurological illness<sup>42</sup>. SARS-CoV-2 is the most recent among a string of Coronavirus epidemics, which early indications suggest that due to its high infectivity, rates of asymptomatic infection, significant incubation time, our relatively limited knowledge of transmission dynamics and overall lack of global pandemic preparation, has evolved into a true global pandemic and has caused significant impact on global health, society and economy.

The severity of this pandemic is driving increased research and funding in all associated areas. One area is on the tracking and epidemiological studies of SARS-CoV-2 infections. One area of focus is in the use of regional wastewater systems, where the compartmentalisation of these systems offers distinct tracking in real time, without the lag for symptom appearance and clinical diagnosis<sup>43</sup>. In addition, the data collected can be used as a supplemental and low-cost surveillance indicator on the circulation of the virus in a community without the need to screen individuals. Further, it contributes to the tracking of infection prevalence, by adding another epidemic indicator<sup>44</sup>.

RT-PCR is the standard method to test for SARS-CoV-2, but samples typically require concentration and removal of non-Coronavirus material prior to testing to ensure optimal results. Ultrafiltration is a successful method for this<sup>43</sup>, and some examples have been given in Table 6.

**Table 6: Summarized examples of ultrafiltration applications using Sartorius Vivaflow® and Vivaspin® for virus and viral RNA in wastewater samples**

Goal of Research (Type of virus, Host Organism)	Purpose of Filtration (Buffer System)	Sartorius Ultrafiltration Device (MWCO)	Subsequent Step	Ref.
Measurement of SARS-CoV-2 RNA in sewage	Concentration of viral RNA	Vivaspin®, PES (50 kDa)	Viral RNA extraction and purification RT-qPCR quantification	43, 44, 45
Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater	Concentration of viral RNA	Vivaspin®100, PES (10 kDa)	Viral RNA extraction and purification RT-qPCR quantification	46
Evaluation of two methods to concentrate SARS-CoV-2 from untreated wastewater	Concentration of viral RNA from 40 ml (total) to 700-1000 µl	Vivaspin® (10 kDa)	Viral RNA extraction and RT-qPCR and ddPCR quantification	47
Virus detection in full scale membrane bioreactor (MBR) plant by virus concentration monitoring, inc. Norovirus, Sapovirus and Rotavirus	Concentration of viral particles in effluent	Vivaflow® 50, PES	PEG precipitation Viral RNA quantification	48
Evaluation of membrane bioreactor wastewater virus removal, inc. Norovirus, Sapovirus, Adenovirus	Concentration of effluent from 1 L to 40 mL	Vivaflow® 50, PES	Nucleic acid extraction RT-PCR quantification	49
Evaluation of membrane bioreactor wastewater Norovirus removal	Concentration of viruses in effluent	Vivaflow® 50, PES	Nucleic acid extraction RT-PCR quantification	50
Evaluation of the association between number of hepatitis E cases in the community and concentration in local sewage	Concentration of viruses in effluent	Vivaflow® 50, PES	Nucleic acid extraction RT-qPCR quantification	51

## Conclusion

The purification of virus by ultrafiltration is virtually independent of the chemical properties and the structure of the virus particles. As viruses have a size within the range of about 20 nm up to several hundred nanometers, they are typically several orders of magnitude bigger than even the largest protein complexes.<sup>7</sup> Therefore, most viruses are unfailingly retained on membranes with MWCOs up to 1,000 kDa. The exact specifications of the ideal ultrafiltration membranes depend on the purpose of the subsequent application.

Ultrafiltration for the concentration of Coronavirus species plays an important role in a range of workflows. Perhaps due to the size distribution of viruses and VLPs, the exact MWCO used is not standard across each study. Although typically, for 80–120 nm particles the 100 kDa MWCO would provide the optimal balance between recovery, removal of interfering substances, speed and shear stresses. Whereas for the recovery of RNA material, lower MWCOs (10–50 kDa) are recommended to capture a greater range of RNA chain lengths. However, until further standardization is confirmed for each application, it is prudent to test specific devices before implementing into procedures.

During the preparation of viral vectors for medical studies, a buffer exchange after column purification can be performed with various MWCOs of all sizes.<sup>8,9,10,15</sup> To separate virus particles from small proteins, a 1,000 kDa cut off has been shown to work.<sup>12</sup> For the complete removal of HCV from blood serum a 30 kDa MWCO has been utilized.<sup>11</sup> When the assessment of whole virus content is crucial (e.g. food, drinking water or marine water samples) smaller MWCOs (5–100 kDa) are used to ensure full recovery of virus particles.<sup>19,21,22,25–29</sup>

## Abbreviations

AAV	Adeno-associated virus
CNS	Central nervous system
	DNA Deoxyribonucleic acid
CoV	Coronavirus
ELISA	Enzyme-linked immunosorbent assay
FPLC	Fast protein liquid chromatography
fCoV	Feline Coronavirus
hCoV	Human Coronavirus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
kDa	Kilodalton (1,000 g per mole)
M	Molarity (mole per litre)
MERS	Middle east respiratory syndrome
mol	Mole
MWCO	Molecular weight cut-off
nsp	Nonstructural protein
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PEG	Polyethylene Glycol
PES	Polyethersulfone
RNA	Ribonucleic acid
SARS	Severe acute respiratory syndrome
RBD	Receptor binding domain
BLI	Bio-Layer Interferometry
RT-PCR	Reverse transcriptase-polymerase chain reaction
ddPCR	Droplet digital polymerase chain reaction
TCIDP50	50% Tissue culture infective dose
VLP	Virus-like particle

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## 5. Sartorius Ultrafiltration Products in the Preparation of Biological Nanoparticles and Medical Nanocarriers



### Introduction

Paul Ehrlich was inspired by the idea of the “magic bullet”<sup>\*</sup> when he for the first time described in theory toxic drugs assembled to so-called “Nanocarriers” in 1908.<sup>1</sup> Today, Nanocarriers have found multiple applications in modern medicine and biotechnology. A key application for these special nanomaterials is a targeted delivery of drugs where they act as transport modules (i. e. as nanoparticles, vesicles, or micelles) for the active ingredient.<sup>2,3,4,5</sup> This is assumed to be more effective and less toxic to the (human) organism compared to traditionally administered drug substances.<sup>6</sup> Besides drug delivery, various further fields using Nanocarriers evolved during the last decades; e. g. magnetic resonance imaging or stem cell gene therapy with metal-based nanoparticles,<sup>7,8</sup> or optical imaging with quantum dots.<sup>9</sup>

Nanocarriers can be categorized by their starting material (i. e. metal-, lipid-, polymer-, and protein-based) and by their formation after preparation (i. e. vesicles, particles and micelles). In general, the preparation of a nanoparticle suspension or a vesicle dispersion in an aqueous medium consists of three steps: a) assembly of the Nanocarriers (for example, by injections, film hydration, or reverse phase evaporation), b) purification (for example, by chromatography, dialysis or ultrafiltration), and c) concentration (for example, by ultrafiltration or evaporation).

This short review provides examples of recent literature dealing with the preparation of Nanocarriers. Particular focus is laid on the concentration and purification steps which were performed via ultrafiltration with Sartorius VivaSpin<sup>®</sup> or VivaFlow<sup>®</sup> devices with different pore sizes (respectively

molecular weight cut-off, MWCO). The VivaSpin<sup>®</sup> portfolio spans a volume range from 0.1 to 20 mL, whereas the VivaFlow<sup>®</sup> system covers volumes from 0.1 to 5 liters. Thus, Sartorius offers an unrivaled wide range of processable sample volumes, membrane materials and MWCOs to meet the different requirements of their intended use. Challenges in this context are buffer exchange after synthesis, desalting and washing,<sup>10,11</sup> exclusion of solubilized compounds,<sup>12,13,14</sup> or aggregates.<sup>15</sup>

Purification is essential to obtain isotonic conditions for in vivo applications, to prevent aggregation or agglomeration and to remove free toxic drugs, ligands, or other substrates potentially triggering side effects. Concentration steps are essential to adjust the amount of pharmaceutical active ingredient in the drug and achieve the anticipated therapeutic or diagnostic effect.

During purification, the separation of free substances (starting material) from the desired Nanocarriers via size-exclusion chromatography (SEC) leads to an unavoidable dilution and to the necessity of a subsequent concentration step. In contrast, dialysis purifies without significant dilution but a concentration step can still be mandatory, if higher Nanocarrier concentrations are necessary. Both separation methods require quite extensive, costly and timeconsuming manual handling. This drawback is overcome with the ultrafiltration utilized by centrifugation in VivaSpin<sup>®</sup> or with a peristaltic pump for the VivaFlow<sup>®</sup> system. This technique is less expensive and quickly performed with very little manual input. Noteworthy is that purification and concentration steps are performed simultaneously.<sup>16</sup>

<sup>\*</sup> In German “Zauberkegel”, opera “Freischütz” by Carl Maria von Weber

After the Nanocarrier is purified, the determination of drug loading (conjugation or encapsulation efficiency) is commonly performed. The conjugation or encapsulation efficiency is one of the reference values to describe and characterize Nanocarriers. Other important properties are the zeta potential and the size distribution determined via photon correlation spectroscopy (PCS), high-resolution transmission electron microscopy (HRTEM) imaging, or dynamic light scattering (DLS).

Prior to performing these different characterizations, a successful purification and concentration of the suspension or dispersion is essential.

In the following tables you can find an overview of publications using ultrafiltration steps for the purification and concentration of different kinds of Nanocarriers. Table 2 provides guidance on which devices and MWCOs to use.

**Table 1 summarizes examples of Nanocarrier ultrafiltration applications with Sartorius Vivaspin® or Vivaflow®:**

Nanocarrier: Nanoparticle, Vesicle, Micelle	Size distribution obtained via (HR)TEM or DLS, Z-Average via PCS and others-if reported	Application	Ref.
<b>Nanoparticles from metal, metal oxides and functionalized metals</b>			
Iron oxides nanoparticles with cisplatinbearing polymer coating	SD: 4.5 ± 0.9 nm via X-Ray-Diffraction Analysis	Magnetic resonance imaging	7
Functionalized iron oxide nanoparticles	SD: 38 and 40 nm via DLS	Stem cell gene therapy and tracking	8
Gold nanoparticles	SD: 0.8 – 10.4 nm via Atomic Force Microscopy	Antimicrobial activity	17
Protein coated gold nanoparticles	SD: 15 and 80 nm via TEM	Drug delivery	18
Functionalized gold nanoparticles	Core-SD: 2 nm via TEM	Targeted imaging tool and antigen delivery	19
Functionalized gadolinium-based nanoparticles	Z-Average: 1.1 ± 0.6 nm and 4 – 14 nm	Diagnostic and therapeutic application	20, 21
Functionalized nanocrystals	10 to 20 nm	Quantum dots for imaging	9
<b>Nanoparticles from polymers, functionalized polymers and polymersomes</b>			
Polymer based Nanoparticles		Drug delivery	22
Curdlan coated polymer nanoparticles	Z-Average: 280 – 480 nm depending on the composition	Macrophage stimulant activity and drug delivery	23
Docetaxel-carboxymethylcellulose Polymer Nanoparticles	Z-Average: 118 ± 1.8 nm	Anti-cancer efficacy studies	4
Functionalized Polymersomes	Z-Average: 185 nm	Surface functionalization studies	3



<b>Lipid Nanoparticles and Liposomes</b>			
Liposomes and micelles	Z-Average: 100 nm for Liposomes and 15 nm for micelles	Ischemia-reperfusion injury	25
Solid lipid Nanoparticles	Z-Average: 100 – 120 nm depending on the used lipid	Drug delivery (Brain Targeting)	26
Bacterial outer membrane vesicles	SD: 124 nm via TRPS	Tunable resistive pulse sensing (TRPS) Analysis	27
Bacterial outer membrane vesicles		Basic research	28
Bacterial outer membrane vesicles	SD: 95 nm	Basic research	29
Bacterial outer membrane vesicles	SD: 50 – 150 nm via TEM	Basic research	30
Liposomes		Drug delivery	2
Liposomes		Encapsulated hydrophilic drugs (Drug delivery)	31
<b>Micelles</b>			
Micelles		Drug delivery	4
Hydrophobic drug micelles based on polymers	SD via DLS: 39 – 165 nm depending on compound in use	Drug delivery	14
<b>Protein Nanoparticles</b>			
Protein Nanoparticles	SD: 20 – 40 nm via DLS	Drug carrier studies	32

SD = Size distribution

**Table 2 lists example Sartorius devices and typical MWCOs used for each nanocarrier ultrafiltration application:**

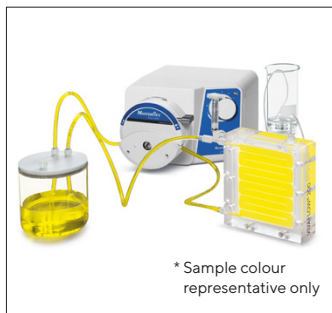
Nanocarrier: Nanoparticle, Vesicle, Micelle	Sartorius Ultrafiltration Device	MWCO	Ultrafiltration purpose	Ref.
<b>Nanoparticles from metal, metal oxides and functionalized metals</b>				
Iron oxides nanoparticles with cisplatinbearing polymer coating	Vivaspin® 20	100 kDa	Purification and concentration	7
Functionalized iron oxide nanoparticles	Vivaspin® 20	100 kDa	Washing step	8
Gold nanoparticles	Vivaspin® 20	5 kDa	Purification step	17
Protein coated gold nanoparticles	Vivaspin® 6	10 kDa	Separation of Nanoparticles   Dyes and washing	18
Functionalized gold nanoparticles	Vivaspin®	10 kDa	Purification step	19
Functionalized gadolinium-based nanoparticles	Vivaspin®	5 kDa and 10 kDa	Purification and concentration	20, 21
Functionalized nanocrystals	Vivaspin®	300 kDa and 50 kDa	Separation of quantum dots-antibody conjugates from starting material (prior to enumeration)	9
<b>Nanoparticles from polymers, functionalized polymers and polymersomes</b>				
Polymer based Nanoparticles	Vivaspin®	30 kDa	Purification and concentration	22
Curdlan coated polymer nanoparticles	Vivaspin® 20	3 kDa	Washing	23
Docetaxel-carboxymethylcellulose Polymer Nanoparticles	Vivaspin®	10 kDa	Concentration	4
Functionalized Polymersomes	Vivaspin® 20	10 kDa	Concentration	3
<b>Lipid Nanoparticles and Liposomes</b>				
Liposomes and micelles	Vivaspin® 20	100 kDa	Concentration	25
Solid lipid Nanoparticles	Vivaflow® 50	100 kDa	Purification	26
Bacterial outer membrane vesicles	Vivaflow® 200	100 kDa	Buffer exchange and concentration	27
Bacterial outer membrane vesicles	Vivaspin® 500 and 20	100 kDa	Buffer exchange and concentration	28
Bacterial outer membrane vesicles	Vivaflow® 200	100 kDa	Buffer exchange and concentration	29
Bacterial outer membrane vesicles	Vivaspin®	100 kDa	Buffer exchange and concentration	30
Liposomes	Vivaspin®	100 kDa	External buffer exchange	2
Liposomes	Vivaflow® 50	100 kDa	Elimination of the free drug	31
<b>Micelles</b>				
Micelles	Vivaspin®	30 kDa	Separation of free substrate and concentration	4
Hydrophobic drug micelles based on polymers	Vivaflow®		Surfactant removal	14
<b>Protein Nanoparticles</b>				
Protein Nanoparticles	Vivaspin® 500	3 kDa	Separation of the free from the encapsulated drug (Drug binding quantification by subsequent UV-vis analysis)	32

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## 6. Vivaflow® and Vivaspin® Workflow in Protein Research Laboratories



### Introduction

Efficiency and efficacy of a multiple cycle experimental procedure was performed using Vivaflow® tangential flow cassettes for initial concentration and diafiltration of a cell culture supernatant. This was followed by Vivapure® Ion Exchange spin columns for the protein purification step and finally Vivaspin® 20 ultrafiltration devices for the final sample concentration and desalting. An artificial mixture of proteins in a RPMI-1640 culture medium was created to mimic the type of product that many researchers culture using e.g. the UniVessel device. This procedure further reflects a method that can be adapted to a large number of protein purification protocols, selecting alternative MWCOs and device sizes where necessary.

### Methods

#### Part 1 – Creating and concentrating the culture medium

2 bottles (4 g) of RPMI-1640 were dissolved into 1.8 L dd-H<sub>2</sub>O and 4 g of sodium acetate was added.

The pH was adjusted to 7.2 using 1M HCl. 2 g of BSA and 1 g of Lysozyme was added as protein samples, meant to be separated by chromatography. The volume of the cell culture supernatant sample was brought up to 2 L using dd-H<sub>2</sub>O. After every preparation, concentration and purification step, 1 mL sample was set aside for SDS gel analysis.

Ion exchange chromatography was the method of choice for purifying lysozyme from the cell culture supernatant, especially from the “contaminant” BSA. For this, the 2 L cell culture supernatant needed to be concentrated and then diafiltered to adjust the sample to the starting conditions needed for the ion exchange chromatography binding step.

For concentration and diafiltration, the Vivaflow® 200 was used with a 5 kDa MWCO PES membrane. Vivaflow® 200 is a ready-to-use laboratory cross-flow cassette in an acrylic housing, which allows caustic cleaning and 4–5 re-uses. Two cassettes can be run in parallel for the concentration of up to 5 L sample volumes. For the 2 L sample to be concentrated in this experiment, one cassette was sufficient. A Masterflex pump with an Easy-Load, size 16 pump head was used to run the Vivaflow® 200 cassette. Figure 1a. and 1b. show the Vivaflow® 200 set up with one or two cassettes.



The Vivaflow<sup>®</sup> 200 system was operated at 3 bar. Once 1.8 L of filtrate had been collected, the pump was stopped, the tubes removed from the cell culture medium concentrate and filtrate and the Vivaflow<sup>®</sup> system was purged with dd-H<sub>2</sub>O. This solution now contained a 10-fold concentration of the constituent proteins from the original culture medium.

A BCA protein detection assay conveyed a 100% recovery of protein after this first concentration step. Table 1 indicates the time needed for the sample concentration.

Filtrate Volume (mL)	Time (hr:min:sec)
0	0:00:00
100	0:03:16
200	0:06:50
300	0:10:45
400	0:14:38
500	0:18:36
600	0:22:43
700	0:26:57
800	0:31:14
900	0:36:01
1,000	0:40:50
1,100	0:45:46
1,200	0:50:36
1,300	0:55:32
1,400	1:00:24
1,500	1:05:26
1,600	1:10:28
1,700	1:15:52
1,800	1:21:50

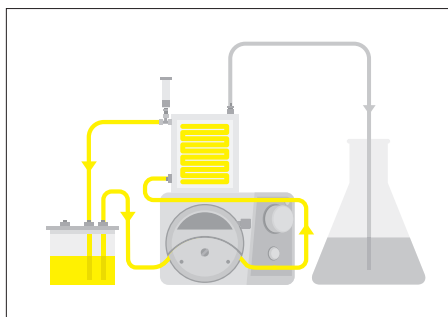


Figure 1a. Vivaflow<sup>®</sup> 200 – single cassette

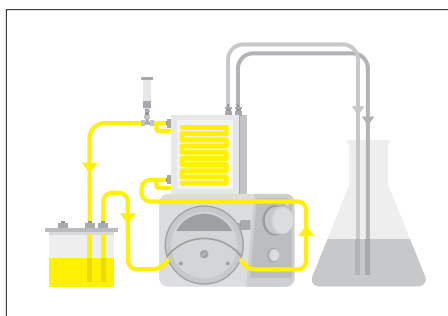


Figure 1b. Vivaflow<sup>®</sup> 50R – two cassettes

Table 1: Vivaflow<sup>®</sup> 200, 5 kDa MWCO PES concentration speed

## Part 2 – Buffer exchange of culture medium concentrate

The Vivaflow® 200 system was used for fast and easy diafiltration. To this end, the diafiltration reservoir, a Vivaflow® accessory, was filled with the 200 mL concentrated sample. Figure 2 shows the diafiltration set up. The Vivaflow® 200 system was set up as before, however attaching an additional tube to the diafiltration lid and placing this new inlet tube into a 25 mM sodium acetate (pH 5.5) buffer (needed to re-adjust the sample concentrate to the ionic starting conditions of the ion exchange chromatography step which was to follow). This enables concentration of the sample in the reservoir to the extent that the original buffer is removed and collected as waste (filtrate). Simultaneously, new buffer (25 mM sodium acetate) is drawn into the closed system, gradually leading to a buffer exchange while keeping the sample volume constant at 200 mL. The system was run at 3 bar. Once 1 L of buffer had been exchanged, diafiltration was stopped.

The 200 mL solution now contained the correct buffer to maintain the stability of the proteins of interest for the next part of the protocol and had the correct pH and salt concentration for the ion exchange binding step. BCA protein quantification again showed a 100% protein recovery.

Table 2 shows the time needed for diafiltration of 200 mL sample against 1,000 mL exchange buffer, again using Vivaflow® 200 with a 5 kDa MWCO PES membrane.



Figure 2: Diafiltration system set up for buffer exchange. Culture medium concentrate can be seen in the center of the image. 1 L 25 mM sodium acetate (exchange buffer) can be seen connected to the system on the left of the image.

Filtrate Volume (mL)	Time (hr:min:sec)
0	0:00:00
100	0:06:58
200	0:14:16
300	0:22:39
400	0:29:40
500	0:37:02
600	0:44:15
700	0:51:34
800	0:58:54
900	1:06:03
1,000	1:13:02

Table 2: Diafiltration of 200 mL concentrated cell culture supernatant containing the proteins lysozyme and BSA against 1,000 mL 25 mM sodium acetate.

### Part 3 – Purification of Lysozyme, the protein of interest

The purification of Lysozyme was performed using a Vivapure® cation exchange membrane adsorber device (Vivapure® Maxi H S). The membrane adsorber matrix holds the active ligands and performs like a traditional cation exchanger. However, membrane adsorbers represent a special form of chromatography matrix. Unlike traditional resins, they make use of convective transport to bring proteins to the ion exchange surface; hence, binding, washing and elution is performed quickly and high binding capacities are achieved, even at high flow rates. This allows their use in fast and convenient centrifugal spin columns (Figure 3).

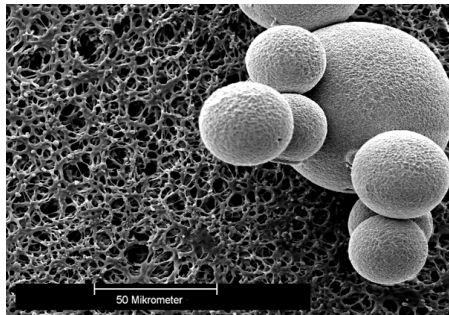


Figure 3: Electron micrograph of chromatography gel beads (upper right) in comparison to a Q ion exchange membrane adsorber (background), revealing 100-fold larger pore sizes of the membrane adsorber.

Two Vivapure® Maxi H S type devices (Figure 4) were each equilibrated with 10 mL of 25 mM sodium acetate (pH 5.5), by filling with 10 mL of this buffer and centrifuging for 5 min. in a swing bucket centrifuge at 500 g and discarding the flow through. Using the concentrated and buffer exchanged sample from Part 2, 10 mL samples were pipetted into each of the equilibrated Vivapure® devices and centrifuged again for 5 min. in a swing bucket centrifuge at 500 g. The Vivapure® devices were washed with 10 mL of 25 mM sodium acetate, discarding the flow through, followed by an elution step with 5 mL of 1 M NaCl in 25 mM sodium acetate. A BCA assay revealed 95% lysozyme recovery.



Figure 4: Vivapure® Maxi spin columns can be used in a centrifuge for fast and easy protein purification.



The eluate was then concentrated in a Vivaspin® 20 (5 kDa MWCO PES), Figure 5, and centrifuged at 5,000 g for 10 min. or until approximately 2 mL of concentrate had been collected. The device was then re-filled with 18 mL 50 mM potassium phosphate buffer (pH 7.2) to 20 mL for a final buffer exchange and desalting of the purified sample. The sample was again centrifuged until a final sample volume of 2 mL had been attained. A BCA assay revealed 97% lysozyme recovery.



Figure 5: Vivaspin® 20 ultrafiltration device, on the right with a pressure cap which allows pressurization of the device as an alternative to the regular centrifugal operation.

#### Part 4 – Analyzing the samples

The samples of the individual steps were analyzed by SDS gel, using reducing sample buffer (prepared by adding 50 µL 2-mercaptoethanol to 950 µL Laemmli sample buffer). For all steps, 5 µL of the 1 mL sample taken during the experiment were diluted with 95 µL reducing sample buffer, of which 20 µL were loaded onto a 12% Tris-HCl SDS gel (Figure 6).

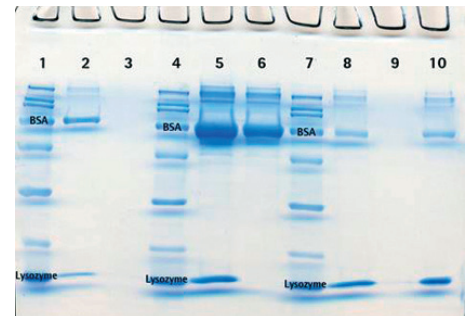


Figure 6: Coomassie stained 12% Tris-HCl SDS gel loaded with 20 µL sample preparations. Lane 1: Marker (SDS Broad Range); Lane 2: Original sample; Lane 3: Original sample filtrate (Part 1); Lane 4: Marker; Lane 5: Buffer exchange concentrate (Part 2); Lane 6: Filtrate after binding (Part 3); Lane 7: Marker; Lane 8: Filtrate after elution (Part 3); Lane 9: Filtrate after concentrating and desalting (Part 3); Lane 10: Concentrate after concentrating and desalting.

## Conclusion

The overall result shows that a standard and straightforward procedure can be followed to concentrate, purify, isolate and analyze a protein of interest from a cell culture, using Vivaflow® 200 tangential flow units for cell culture supernatant concentration and diafiltration, Vivapure® for ion exchange chromatography, followed by Vivaspin® 20 for final sample concentration and desalting.

In many cases dialysis, which is an overnight procedure would be performed instead of the much quicker alternative - ultrafiltration. Here, we show how time-saving and efficient ultrafiltration is for diafiltration and desalting applications, as well as for protein concentration.

The set up and completion of protein purification takes approx. 3.45 h using this method, starting from a culture supernatant, with high protein recoveries in each step (see Table 3). A total protein purification procedure can therefore be completed within 1 working day, including SDS gel analysis, utilizing this strategy, when adapted to individual needs.

Task	Time	Recovery
Vivaflow® 200 set up and concentration	1 hour 25 min.	100%
Vivaflow® 200 set up and diafiltration	1 hour 20 min.	100%
Vivapure® purification	45 min.	95%
Vivaspin® concentration and desalting	30 min.	97%
Total	3 hours 45 min.	92%

Products used in this experiment	Order No.
Vivaflow® 200, 5 kDa MWCO PES	VF20P1
500 mL Diafiltration Reservoir	VFA006
Vivapure® S H Maxi	VS-IX20SH08
Vivaspin® 20, 5 kDa MWCO PES	VS2011

Table 3: Processing times for a complete protein purification workflow.




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