Lab Ultrafiltration

Tips and Tricks

Simplifying Progress

SARTORIUS

Build Knowledge Through Experiments

The use of ultrafiltration membranes for concentration and purification of proteins and DNA is ubiquitous in biological laboratories. Filter devices with ultrafiltration membranes can also be used for concentration of other macromolecules such as extracellular vesicles, viruses, and inorganic polymers and nanoparticles. Although performing sample concentration and buffer exchange using an ultrafiltration device is relatively simple, some tricks of the trade can improve target molecule recoveries and speed up your workflow considerably.

The following Application Notes provide an overview of how to:

Desalt or re-buffer samples

Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators

Define final concentrate volumes

Concentration to a Defined Final Volume with Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES

Improve target recovery

Treatment of Vivaspin® Concentrators for Improved Recovery of Proteins at Low Initial Concentration

Minimize endotoxin levels

Depyrogenation of Vivaspin® Turbo 15 PES in Comparison to Ultrafiltration Devices With a Regenerated Cellulose Membrane

Use Picus® with Vivaspin®

Effectively Use Picus® Pipettes with Vivaspin® Concentrators

Perform continuous diafiltration

Vivaspin® 20 Diafiltration Cups: A Rapid Alternative to Buffer Exchange by Dialysis

SARTORIUS

Application Note

April 03, 2019

Keywords or phrases:
Diafiltration, buffer exchange, desalting

Desalting and Buffer Exchange with Vivaspin® Centrifugal Concentrators

Pieter Eyckermann[2], Rik McRae[1], Andreas Kocourek[2], Robert Zeidler[2] and Adam Green[1]

1. Sartorius Stedim Lab Ltd, Sperryway, Stonehouse, Gloucestershire, GL10 3UT, UK

2. Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Göttingen, Germany

Correspondence
E-Mail: john.cashman@sartorius.com

Abstract

This short application note highlights the ability to reduce protein sample salt concentrations by up to 99%, or to exchange the buffer sample entirely, using Vivaspin® 20 and Vivaspin® 6 centrifugal ultrafiltration devices. This process is known as diafiltration and prevents the over-concentration of proteins with a tendancy to precipitate at higher salt concentration. Furthermore, in comparison to conventional re-buffering techniques such as dialysis, a complete diafiltration process can typically be performed in a matter of minutes, instead of 1 - 2 days or more.

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 10x. For example, if a precipitous protein sample is concentrated to 5x then diluted back to the original volume, and this process is repeated a further two times, this still results in a >99% reduction in salt concentration, without over-concentrating the protein.

Methods

Select an appropriate MWCO for your sample.
For maximum recovery, select a MWCO ⅓ to ½ 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.

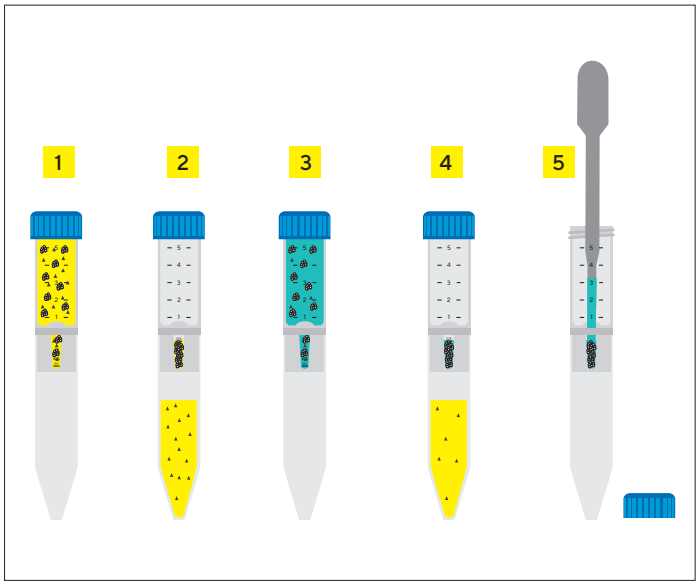


Figure 1: Step-by-step method for desalting and concentration

* 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.

SARTORIUS

Application Note

January 15, 2018

Keywords or phrases:
Concentration ratio, final volume adjustment

Concentration to a Defined Final Volume with Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES

Rik McRae[1], **Hannes Landmann**[2,*]

1. Sartorius Stedim Lab Ltd, Sperryway, Stonehouse, Gloucestershire, GL10 3UT, UK

2. Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Goettingen, Germany

* Correspondence

E-Mail: john.cashman@sartorius.com

Abstract

This short Application Note describes how you can use Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES to concentrate samples to defined final volumes. By adding a particular volume of water or buffer to the filtrate vessel prior to concentration, the dead-stop of the device is effectively increased, enabling accurate control of the final concentrate volume.

Introduction

It is sometimes desirable to be able to preselect a defined final volume for a concentration step, especially when parallel concentrations are being performed. Vivaspin® centrifugal concentrators have a built-in dead-stop feature, which prevents concentration to dryness. Due to the fast concentration rates possible with the patented vertical membrane design in the Vivaspin®, the drying out of the sample would otherwise be a possibility.

Here, we describe a method for achieving reproducible defined final volumes using Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES and Vivaspin® 500 PES centrifugal concentrators. The method does not rely on the dead-stop pocket but instead increases the retentate volume by adding liquid to the filtrate vessel prior to centrifugation.

Equipment

- Vivaspin® Turbo 15 PES 10 kDa MWCO
- Vivaspin® Turbo 4 PES 10 kDa MWCO
- Vivaspin® 500 PES 10 kDa MWCO
- Tacta® 5 mL mechanical pipette and Optifit pipette tips
- Tacta® 1000 µL mechanical pipette and Optifit pipette tips
- Tacta® 200 µL mechanical pipette and Optifit pipette tips
- Arium® Pro ultrapure water system
- Sartorius Precision Lab Balance
- Centrisart® D-16C Centrifuge with swing out rotor for 50 mL and 15 mL falcon tubes
- Centrisart® A-14C Centrifuge with fixed angle rotor for 24 1.5 | 2.2 mL tubes

Reagents

1 mg/mL Bovine Serum Albumin labelled with Bromophenol blue

Methods

1. Add defined amount of water to the filtrate tube (see table).
2. Assemble concentrator insert into the filtrate tube and add sample solution.
3. Close the concentrator screw cap (for Vivaspin® Turbo 15 PES or Vivaspin® Turbo 4 PES) or close the cap (Vivaspin® 500 PES) and place in the centrifuge.
4. Centrifuge to concentrate the sample.
5. Remove the concentrator and recover the concentrated sample with a pipette.

Results

Results for Vivaspin® Turbo 15 PES

Volume of water added to the filtrate tube	Volume of sample added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
11.5 mL	15 mL	20 min @ 4,000 *g*	1.50 ± 0.02 mL
9.5 mL	15 mL	20 min @ 4,000 *g*	0.96 ± 0.01 mL
7.5 mL	15 mL	20 min @ 4,000 *g*	0.53 ± 0.02 mL

Results for Vivaspin® Turbo 4 PES

Volume of water added to the filtrate tube	Volume of sample added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
2.0 mL	4 mL	20 min @ 4,000 *g*	0.34 ± 0.03 mL
1.5 mL	4 mL	20 min @ 4,000 *g*	0.15 ± 0.02 mL
1.2 mL	4 mL	20 min @ 4,000 *g*	80 ± 10 µL

Results for Vivaspin® 500 PES in 40° fixed angle rotor

Volume of water added to the filtrate tube	Volume of sample added to the concentrator insert	Spin conditions	Final concentrate volume (average of 8 devices)
500 µL	500 µL	15 min @ 15,000 *g*	103 ± 13 µL
380 µL	500 µL	15 min @ 15,000 *g*	51 ± 11 µL
250 µL	500 µL	15 min @ 15,000 *g*	30 ± 5 µL
200 µL	500 µL	15 min @ 15,000 *g*	23 ± 7 µL

Conclusion

Reproducible, defined final concentrate volumes can be quickly and easily achieved with Vivaspin® Turbo 15 PES, Vivaspin® Turbo 4 PES, and Vivaspin® 500 PES.

SARTORIUS

Application Note

November 05, 2020

Keywords or phrases:
Ultrafiltration, passivation, protein concentration, low-concentration protein samples, protein yield, non-specific binding, adsorption

Treatment of Vivaspin® Concentrators for Improved Recovery of Proteins at Low Initial Concentration

Rik McRae[1,*], Claudia Naumann[2,*], Kristin Hoehne, Robert Zeidler

1. Sartorius Stedim Lab Ltd, Sperryway, Stonehouse, Gloucestershire, GL10 3UT, UK
2. Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Straße 20, 37079 Göttingen, Germany

* Correspondence
E-Mail: john.cashman@sartorius.com

Abstract

Ultrafiltration is a core technology for the concentration of molecules of interest in the laboratory and research setting. As molecule recovery is a key criteria for high performance; optimal membrane, MWCO and device handling must be in place. Further to this, certain techniques can be employed to minimize loss through non-specific adsorption to device housing material and membrane material. This is most important for samples with low starting concentrations in the nanogram to microgram range, where loss through adsorption can have a significant impact on end recoveries. Here we describe these "passivation" techniques that demonstrate increased recovery when used with low starting concentration 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/cm²) and in practice not detectable.

This can increase to 20 – 100 µg/cm² 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 be an effective passivation agent, though the optimal reagent has to be selected for the respective protein and its hydrophilic | hydrophobic characteristics.

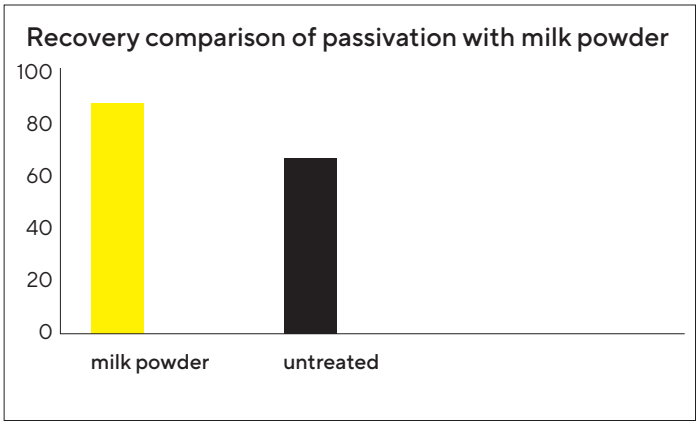


Fig. 1: Protein recovery (10 µg/mL BSA) with Vivaspin® PES 10 kDa after passivation

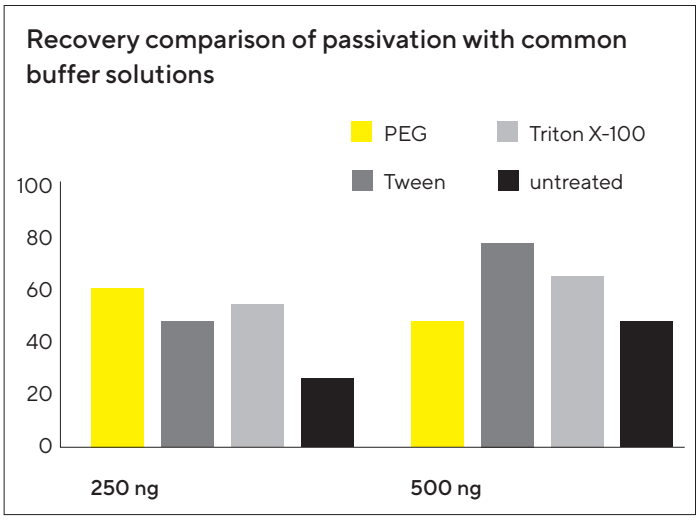


Fig. 2: Protein recovery (250 and 500 ng BSA) with Vivaspin® 2 PES 10 kDa after passivation

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.

Additional application notes can be found on:
https://www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices

SARTORIUS

Application Note

November 12, 2019

Keywords or phrases:
Depyrogenation, protein concentration, ultrafiltration, centrifugal concentrator, polyethersulfone, regenerated cellulose, endotoxins

Depyrogenation of Vivaspin® Turbo 15 PES in Comparison to Ultrafiltration Devices With a Regenerated Cellulose Membrane

Husna Begum[1,*], Mike Brownleader[2,*], Ben Williams[3]

1. Generon Limited, 11 Whittle Parkway, Progress, Business Park, Slough, SL1 6DQ, United Kingdom
2. Generon Limited, 11 Whittle Parkway, Progress, Business Park, Slough, SL1 6DQ, United Kingdom
3. Sartorius Stedim Lab Limited, Sperry Way, Stonehouse Park, Stonehouse, GL10 3UT, United Kingdom

* Correspondence
E-Mail: adam.green@sartorius.com

Abstract

The presence of endotoxin contamination in biologics and virus based research and discovery can have harmful impacts on target quality, yields and analytical confidence. A common source of contamination is via contact with common laboratory disposables. These disposables are critical to research and discovery workflows and so methods to remove endotoxin contamination prior to use, without effecting disposable functional performance, are increasing critical, along with using disposables with low starting endotoxin concentrations. Here we demonstrate low endotoxin concentrations within the Vivaspin® Turbo 15 PES devices and show further successful depyrogenation using sodium hydroxide. Benchmarking against an alternative device from another supplier was included to highlight the maintained functional integrity of the Vivaspin® devices and their suitability to this application.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices/centrifugal

Introduction

Endotoxins (or Lipopolysaccharides) are a component of gram-negative bacteria cell wall, an often unwanted impurity in laboratory based research due to their inflammatory and pyrogenic effect on mammalian immune systems.

Here, the background levels of endotoxin from manufacturing are quantified in both Vivaspin® Turbo 15 PES devices and 15 mL ultrafiltration devices from another supplier (Supplier A). Additionally, both types of devices were subjected to treatment of 1 N NaOH, which is commonly used in laboratories as a basic chemical for depyrogenation. A protocol describes the depyrogenation of Vivaspin® Turbo 15 PES for applications where the absence, thus removal of endotoxin is of critical importance.

Method

A) Analysis of typical baseline endotoxin level

1. 2 × Vivaspin® Turbo 15 PES (10 kDa PES membrane) and 2 × 15 mL UF device, Supplier A (10 kDa regenerated cellulose membrane) were selected.
2. Each device was filled with 15 mL HyPure water and left to stand at 20°C for 30 min.
3. Each device was centrifuged at 3,000 *g* for 10 min until approximately 0.5 mL of concentrate remained (approx. 30-fold) in the deadstop pocket.
4. Samples were retrieved from the filtrate reservoir and loaded onto an Endosafe-PTS cartridge for EU/mL quantification.

B) Effect of NaOH treatment on flux and recovery

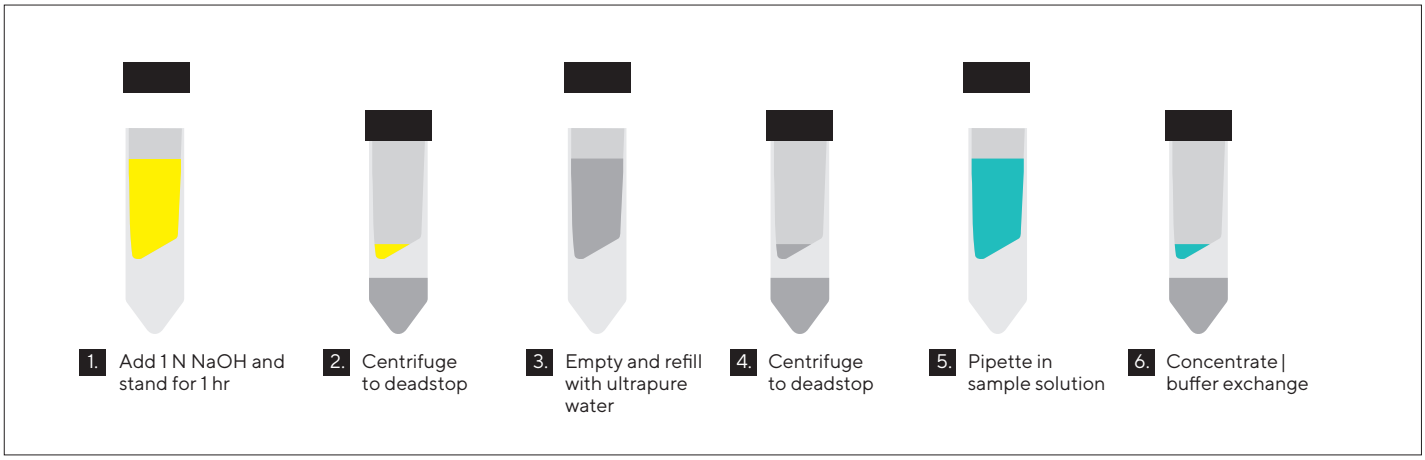
1. 4 × Vivaspin® Turbo 15 PES (10 kDa PES membrane) and 4 × 15 mL UF device, Supplier A (10 kDa regenerated cellulose membrane) were selected.
2. Each device was filled with 15 mL 1 N NaOH and left to stand at 20 °C for 1 hr.
3. Each device was then centrifuged at 3,000 *g* until the device deadstop volume was reached.
4. The devices were emptied, then re-filled with 15 mL HyPure water for the 1st wash cycle.
5. The devices were then centrifuged at 3,000 *g* until the deadstop volume was reached.
6. A 2nd wash cycle was repeated as above.
7. The same devices were then emptied and filled with 15 mL 1.0 mg/mL BSA in saline.
8. All devices were centrifuged at 3,000 *g* until the final concentrate volume was < 0.5 mL.
9. A recovery measurement was then performed on a spectrophotometer.

Results and Discussion

The typical endotoxin levels were an order of magnitude below the guideline maximum threshold of 0.1 EU/mL for intravenous work with a 20 g mouse, showing the inherent cleanliness of the devices in both the Vivaspin® Turbo 15 PES and the 15 mL UF device, Supplier A, even when untreated (table 1).

Upon treatment with 1 N NaOH, the flow rate and protein retention and recovery value in Vivaspin Turbo 15 remained unaffected (table 3). In contrast, the 15 mL UF device with a regenerated cellulose membrane from Supplier A showed a significant reduction in the filtration rate following the use of high pH 1 N NaOH, despite decreasing pH after each wash cycle (table 2).

The total process time following the depyrogenation protocol (described above in method B) was over twice as fast when using the Vivaspin® Turbo 15 PES compared to the 15 mL UF device, Supplier A (table 3).



Schematic depyrogenation process, followed by sample concentration.

Equipment and Test Samples

- Vivaspin® Turbo 15 PES 10 kDa PES (Sartorius, VS15T01)
- 15 mL UF device, Supplier A
- NaOH (Sigma, S0899)
- NaCl (Sigma, S7653)
- HyPure Cell Culture Grade Water, Endotoxin Free (< 0.005 EU/mL) LAL water (HyClone, SH30529.03)
- Albumin from Bovine Serum (Sigma-Aldrich, 1001430867)
- Genova Spectrophotometer (JENWAY, 1282)
- Megafuge 1.0R Centrifuge (Heraeus instruments, 100000494)
- Standard pipettes and tips

Tables and Figures

Table 1: Both the Vivaspin® Turbo 15 PES and 15 mL UF device, Supplier A presented less than 0.01 EU/mL of endotoxin when untreated and tested with a water wash control.

	Vivaspin® Turbo 15 PES		15 mL UF Device, Supplier A	
	1	2	1	2
Final volume	0.54 mL	0.42 mL	0.75 mL	0.52 mL
Endotoxin level	< 0.006 EU/mL	< 0.005 EU/mL	< 0.005 EU/mL	< 0.009 EU/mL

Table 2: Process time taken when devices centrifuged at 3,000 *g*. Depyrogenated Vivaspin® Turbo 15 PES lead to higher recovery of protein after treatment with NaOH. Additionally, the PES membrane remained unaffected by high pH treatment, leading to a faster total processing time by 135 min compared to the time take by the 15 mL UF device, Supplier A.

Device type	Vivaspin® Turbo 15 PES 10 kDa PES	15 mL UF device, Supplier A 10 kDa Regenerated Cellulose
Average time to concentrate BSA 30 × prior to NaOH treatment	15 min	25 min
Average time for NaOH treatment and 2 wash cycles	90 min	225 min
Average time to concentrate protein 30 × post NaOH treatment	15 min	45 min
Final concentrate volume	0.4 mL	0.25 – 0.3 mL
Recovery percentage	97.0%	84.9%
Total process time	105 min	240 min

Table 3: The pH levels of device filtrates were assayed during each wash cycle to demonstrate that even when the pH level was lowered, the negative effect of NaOH on the flow rate of regenerated cellulose was not reversed. The low endotoxin HyPure water and the filtrate from an untreated device presented a baseline pH of 7.55.

	After NaOH treatment	1st wash cycle	2nd wash cycle
pH of filtrate	13.51	11.03	9.32

Conclusion

For applications, in which the absence of endotoxins is essential, we describe a method for fast and reliable depyrogenration of Vivaspin® Turbo 15 PES devices. Additionally, it could be shown that Vivaspin® Turbo 15 PES has superior performance in both flow rate and recovery compared to Supplier A with a regenerated cellulose membrane, following 1 N NaOH soaking treatment for 1 hr.

SARTORIUS

Practical Guide

May 2021

Keywords or phrases:
Yield Measurement, Unknown Volume Measurement, Centrifugal Concentrators, Picus®, Vivaspin®

Effectively Use Picus® Pipettes with Vivaspin® Concentrators

Joni Åke[1]

1. Product Management, Sartorius Biohit Liquid Handling, Helsinki, Finland

Correspondence
E-Mail: LHinfo.Finland@Sartorius.com

Introduction

Use of centrifugal concentrators for buffer exchange or sample concentration requires optimization of the protocol and correct handling of the device in conjunction with a pipette to ensure:

- Determination of the concentrated sample volume for accurate yield measurement
- Retrieve the full volume of sample in a tall concentrator tube while avoiding sample-to-pipette and pipette-to-sample contamination
- Proper rinsing so that the preservatives used to maintain membrane stability do not elute into the sample leading to anomalous peaks in concentrate analysis

In this practical guide, we provide guidance on the efficient use of Picus® pipettes and extended length pipette tips with centrifugal concentrators.

Use of Manual Mode to Measure Yield

An electronic pipette's manual mode allows the user to control aspiration and dispensing with the adjustment wheel allowing for determination of unknown sample volumes. The pipette measures the aspirated volume based on the piston movement, allowing for accurate measurement of an unknown volume by visually controlling aspiration of the sample with the final volume displayed on the pipette. As the concentrate volume is needed to define the protein yield, use of the manual mode ensures the volume is measured accurately when emptying the vessel.

The main factors affecting accuracy of this method are the attentiveness of the user and resolution of the pipette, defined as the smallest volume that can be adjusted; for the resolution of the Sartorius Picus® 1000 µl pipette is 1 µl. Inaccuracy can be introduced by accidentally aspirating air, by not aspirating close to the liquid surface, and small amounts of residual sample left in the sample tube. Step-by-step instructions for using this mode are provided below.

Select Suitable Pipette Tips

Reaching liquid inside a tall tube can be challenging while simultaneously avoiding contamination. As such, use of the correct type of pipette tips and concentrators with angular dead stops will ensure complete recovery of the concentrate. If the pipette shaft enters the tube, there is a risk of contamination. Extended length pipette tips are therefore recommended as their length eliminates the need for the pipette to be brought into the concentrator.

Use of low retention tips can help maximize sample recovery with liquids with low surface tension which tend to leave a film of liquid on the inner surface of a standard length pipette tip.

Pipetting Speed

In applications where air bubbles or foam generation are an issue, the low surface tension of low retention pipette tips can help prevent formation of bubbles or foam. Foam and bubbles can also be prevented by adjusting the speed setting of the pipette to a slow speed.

Figure 1. *Pipetting BSA solution (1 mg/ml) with slow speed (Picus® setting 1)*

Figure 2. *Pipetting BSA solution (1 mg/ml) with fast speed (Picus® setting 9).*

Basic Steps for Centrifugal Concentration

1. Prepare Vivaspin® concentrator
    a. For membrane rinsing: in pipetting mode, pipette up and down three to five times to rinse the membrane and remove preservative
    b. For device passivation: pipette the passivation solution up and down three to five times over the membrane and concentrator insert housing and remove the solution (rinsing the membrane and device housing with passivation buffer solutions can reduce any non-specific binding between molecule of interest and membrane or device surface)
2. Perform concentration with centrifuge. Concentration time depends upon desired concentration factor and Relative Centrifugal Force (RCF)
3. Measure volumetric yield with Picus® manual mode

Use of Manual Mode with Picus® Pipettes to Measure Volumetric Yield

1. Set the pipette to manual mode
2. Adjust speed settings to 3 for aspiration and 3 for dispensing; accurate control of the aspiration is easier at a slow speed setting
3. Ensure the tip is below the liquid surface when aspirating while avoiding the tip being placed too close to the bottom as this may interfere with liquid flow and distort results
4. Use the adjustment wheel to aspirate liquid into the tip in a controlled manner; the force with which the wheel is turned determines the aspiration speed (see above for setting the maximum speed)
5. As the liquid is drawn into the tip, ensure the end of tip is close to the bottom of the vessel; the angular dead stop of the Vivaspin® assists with this
6. Release the wheel as soon as all liquid is aspirated into the tip and observe if any residual liquid gathers at the bottom of the vessel; if so, continue aspiration by turning the wheel
7. Read the total volume aspirated from the pipette's display
8. Empty the tip completely by pressing QUIT and double-clicking the operating button

Vivaspin®, Picus®, and Pipette Tip Compatibility

The table below lists the pipette tips that fit various Vivaspin® concentrator tubes allowing for complete aspiration of liquid.

Table 1. *Vivaspin® Compatibility to Tips and Picus® Pipettes*

Vivaspin®	Tip	Picus®
Vivaspin® Turbo 15 RC 5-120 µl	LH-X781000, LH-X781001 LH-XF781001	735081 LH-745081
Vivaspin® Turbo 15 PES	LH-X781000 LH-X781001 LH-XF781001	735081 LH-745081
Vivaspin® Turbo 4 RC	LH-X781000 LH-X781001 LH-XF781001 If concentrate volume is below 100 µl use: LH-X780200 LH-X780201 LH-XF780201	735081 LH-745081 735041 LH-74504
Vivaspin® 20	LH-X781000, LH-X781001 LH-XF781001	735081 LH-745081
Vivaspin® 6	LH-X781000 LH-X781001 LH-XF781001	735081 LH-745081

Summary

Sartorius Picus® and extended length pipette tips enable collection of concentrated samples from Vivaspin® concentrators. Use of the Picus® pipette manual mode enables convenient measure of sample volume and extended length pipette tips ensure that the entire sample can be reached in tall vessels while protecting the sample and pipette from contamination.

SARTORIUS

Application Note

Circa 2016

Keywords or phrases:
Diafiltration, Dialysis, Constant Volume Buffer Exchange

Vivaspin® 20 Diafiltration Cups: A Rapid Alternative to Buffer Exchange by Dialysis

Marlene Völler[1], Hannes Landmann[2], Richard McRae[3], Ben Williams[3], John Cashman[4]

[1] University of Applied Sciences Osnabrück, Albrechtstraße 30, 49076 Osnabrück, Germany
[2] Sartorius Lab Instruments GmbH & Co. KG, Otto-Brenner-Strasse 20 37079 Goettingen, Germany
[3] Sartorius Stedim Lab Ltd., Sperry Way, Stonehouse, Gloucestershire, GL10 3UT UK
[4] Sartorius UK Ltd., Longmead Business Centre, Blenheim Road, Epsom, KT19 9QQ UK

Correspondence
Email: john.cashman@sartorius.com

Abstract

Many typical protein purification workflows will include a buffer exchange of the protein sample. This may be important to provide the appropriate conditions for the next purification step, prepare the protein of interest for use in downstream applications, or ensure stability of the purified protein. A conventional buffer exchange process may be performed by dialysis. However, this method is time consuming, requires large volumes of the exchange buffer, and increases the potential for degradation of the target protein by proteases in the sample. Diafiltration (DF)—a process using ultrafiltration devices for the same purpose—ensures a much faster, effective, and safer buffer exchange. Here, we demonstrate the increased efficiency of buffer exchange when using Vivaspin® 20 centrifugal ultrafiltration devices with DF cups. Unique to Sartorius, these DF cups enable a gradual change to the sample buffer composition, ensuring a gentle but still more efficient buffer exchange.

Find out more: www.sartorius.com

Introduction

During the preparation of biological samples, buffer exchange is an essential step, as it prepares the sample for downstream applications or enables subsequent long-term storage.[1,2] It can be performed by dialysis or diafiltration. Diafiltration with Vivaspin® 20 and Sartorius DF cups is a well-established method in protein science laboratories for buffer exchange and desalting steps. To only highlight a few examples, it has been applied by Read *et al.*[3] in the preparation of fusion proteins for a linking reaction to affinity purification columns. Here, GST fusion proteins were purified by glutathione-agarose affinity chromatography and subsequently the buffer was exchanged to a coupling buffer (0.2 M $NaHCO_3$, 0.5 M NaCl, 2 M urea, pH 8.3) using the DF cup.[4] Aziz *et al.*[5] performed a desalting step prior to crystallization of the receiver domain of a putative response regulator, BPSL0128. Here, 0.2 M NaCl, 50 mM Tris pH 8.0 was exchanged for 10 mM Tris pH 8.0 using the DF cup. Tovar-Herrera *et al.*[6] desalted the expansin protein ScExlx1 prior to activity assays (20 mM NaH_2PO_4, 20 mM imidazole, 0.5 M NaCl pH 7.4 against 50 mM NaOAc, pH 5). Finally, Guccione *et al.*[7] desalted active site subunit of methylmenaquinol: fumarate reductase (Mfr) prior to enzymatic assays (1.5 M NH_4SO_4, 50 mM Tris, pH 8.0 against 50 mM Tris, pH 8.0).

The dialysis process traditionally used for buffer exchange in biological samples relies on passive diffusion. It is therefore time consuming and requires large volumes of dialysis buffer.[8] Here we present an approach based on diafiltration with Vivaspin® 20 centrifugal concentrators. In combination with Sartorius DF cups, these devices offer a fast, efficient, and reliable way to exchange protein sample buffers. The gradual buffer exchange by diafiltration allows for gentle salt removal from protein samples prone to precipitating at high salt concentrations and thus keeps them in solution. In addition, the short processing time helps prevent degradation of the protein of interest by proteases.

Materials and Methods

To assess the effectiveness and performance of diafiltration in comparison to the conventional dialysis approach, Sartorius Vivaspin® 20 products were used in parallel to dialysis cassettes. A dialysis utilizing these cassettes was performed according to the instructions given by the manufacturer, following an overnight procedure. The aim was to reduce the salt concentration by 99%.

The Vivaspin® 20 operating conditions for buffer exchange were optimized with and without a DF cup, using a BSA model solution and CHO cell culture supernatant (salt reduction from 1 M to 0.01 M).

Optimal conditions for > 99% salt reduction were:
- 4,000 *g* in a swing bucket rotor
- 15 mL exchange buffer

Centrifugation time for each sample type was determined (Table 1) for two spins to reach the dead stop volume, with an addition of exchange buffer in between.

Table 1

Centrifugation Times for BSA and CHO With and Without DF Cup

	BSA	CHO
Vivaspin® 20 without DF cup	2 × 8 min	2 × 45 min
Vivaspin® 20 with DF cup	2 × 6 min	2 × 45 min

After optimizing the Vivaspin® 20 desalting conditions, the diafiltration procedure was compared to dialysis. The desalting process was more efficient with Sartorius DF cups.

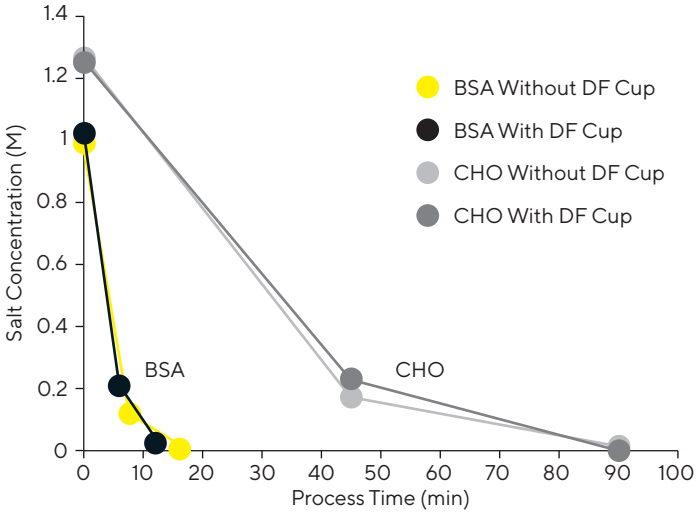
Following the buffer exchange, the integrity of all protein samples was checked by SDS-PAGE. Salt concentrations were assessed by conductivity measurement.

Results

Comparison of Buffer Exchange Using Vivaspin® 20 and Dialysis Cassette

The desalting process was performed using Vivaspin® 20, with and without a DF cup. For this experiment, two samples were used: 2 mL BSA model solution and 2 mL CHO culture supernatant. Figure 1 shows the salt concentration measured for each sample plotted against the time taken to achieve > 99% buffer exchange.

Figure 1
Diafiltration With Vivaspin® 20



Note. Salt concentration during diafiltration in Vivaspin® 20 (30 kDa MWCO) with BSA solution (yellow | black lines; 1 mg/mL solved in 1 M NaCl/0.25 mM NaOAc) and CHO cell culture supernatant (gray lines), deionized water was used as exchange buffer.

Buffer exchange by dialysis using a conventional, pre-assembled dialysis cassette was performed in parallel with the same samples. In accordance with the manufacturer's instructions, the dialysis buffer was changed after 2 hours and 4 hours and the sample was recovered after a final overnight dialysis step. The whole dialysis procedure took approximately 24 hours. In comparison, Vivaspin® 20 devices enable buffer exchange to the desired salt concentration significantly faster than dialysis cassettes (Figures 1 and 2, Table 3).

Comparison of Process Times

The time required for buffer exchange was up to 140 times shorter when using Vivaspin® 20 compared to the method using dialysis cassettes (Table 2 and Figure 2).

Table 2
Time Needed for Each Process Step to Perform Buffer Exchange Using Vivaspin® 20 or Dialysis Cassettes

Buffer Replacement	Vivaspin® 20 Without DF Cup		Vivaspin® 20 With DF Cup		Dialysis Cassette
	BSA	CHO	BSA	CHO	BSA | CHO
1	8 min	45 min	6 min	45 min	120 min
2	8 min	45 min	6 min	45 min	120 min
3	-	-	-	-	1,200 min (20 h)
Total	16 min	90 min	12 min	90 min	1,440 min (24 h)

Figure 2
Comparison of Time Needed for a Complete Buffer Exchange Using Vivaspin® 20 or Dialysis Cassettes

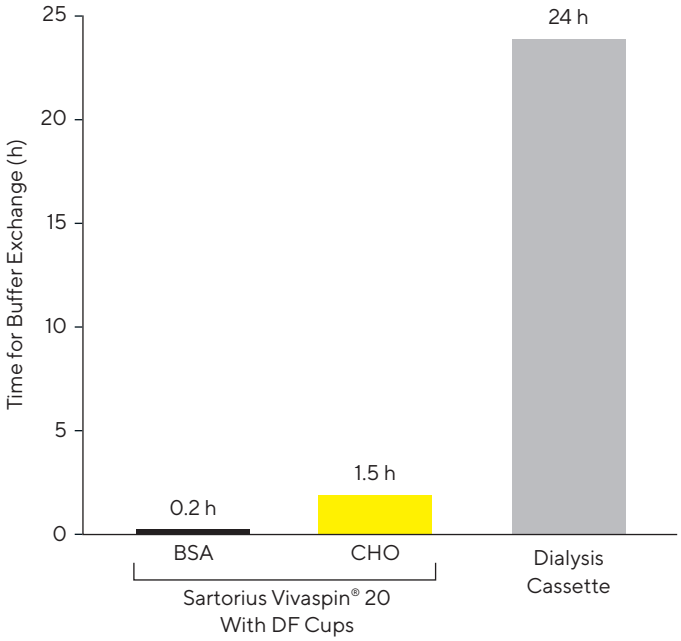


Table 3
Comparison of Salt Concentration Reduction and Process Times for Buffer Exchange of BSA and CHO Cell Culture Supernatant by Diafiltration With Vivaspin® 20 or Dialysis

	Before DF		Diafiltration (DF)			After DF
	Device	Salt Conc.	Buffer Exchange Amount	Hands-On Time	Process Time	Salt Conc. (% original salt conc. remaining)
BSA	Vivaspin® 20 without DF cup	1 M	35 mL	45 min	16 min	0.01 M (0.9%)
	Vivaspin® 20 with DF cup	1 M	30 mL	45 min	12 min	0.01 M (1.6%)
	Dialysis cassette	1 M	1,500 mL	60 min	1,440 min	0.02 M (0.0%)
CHO cell culture supernatant	Vivaspin® 20 without DF cup	1.26 M	35 mL	45 min	90 min	0.02 M (1.83%)
	Vivaspin® 20 with DF cup	1.26 M	30 mL	45 min	90 min	0.01 M (0.95%)
	Dialysis cassette	1.26 M	1,500 mL	60 min	1,440 min	0 M (0%)

Conclusion

Diafiltration using Vivaspin® 20 concentrators allows fast buffer exchange. In combination with the Sartorius DF cups, a gradual buffer exchange can be performed. This gentle buffer exchange ensures a decrease in salt concentration prior to concentration of the target molecule down to the dead-stop volume. This way, proteins prone to precipitation at higher salt concentrations are more likely to remain soluble. The DF cups also help to shorten the process time and allow a more efficient decrease in salt concentration (Figure 1). The spin times should be optimized for each sample by measuring the salt content after each diafiltration step. When the sample is concentrated down to the dead-stop volume prior to each buffer exchange, two spin cycles are typically sufficient to achieve a 99% reduction in salt concentration.

The approach using Sartorius DF cups in Vivaspin® 20 concentrators is superior to traditional dialysis methods due to increased process speed, reduced buffer volume requirements, and ease of use. In contrast, dialysis takes substantially longer and requires more hands-on time. Since buffer exchange with Vivaspin® 20 is much faster, an additional benefit is that the target proteins are largely protected from proteases. Furthermore, dialysis leads to dilution of the sample during buffer exchange and a final concentration step would be necessary to reach the required final concentration. Utilizing Vivaspin® 20 with DF cups enables simultaneous desalting and concentration of the sample and therefore efficiently prevents sample dilution.

Diafiltration with Vivaspin® 20 and DF cups allows for time-efficient recovery of highly concentrated samples in virtually any buffer of choice.

References

1. Cartwright IJ, Higgins JA. Direct evidence for a two-step assembly of ApoB48-containing lipoproteins in the lumen of the smooth endoplasmic reticulum of rabbit enterocytes. *J Biol Chem.* 2001;276(51):48048-57.
2. Grosse C, Grass G, Anton A, *et al.* Transcriptional organization of the czc heavy-metal homeostasis determinant from Alcaligenes eutrophus. *J Bacteriol.* 1999;181(8):2385-2393.
3. Read AJ, Gauci CG, Lightowlers MW. Purification of polyclonal anti-conformational antibodies for use in affinity selection from random peptide phage display libraries: a study using the hydatid vaccine EG95. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009; 877(14-15):1516-1522.
4. Smith DB, Johnson KS. Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. *Gene.* 1988;67(1): 31-40.
5. Aziz AGA, Sedelnikova S, Ruzheinikov SN, *et al.* Crystallization and preliminary X-ray analysis of the receiver domain of a putative response regulator, BPSLo128, from Burkholderia pseudomallei. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* 2012;68(Pt 8):917-922.
6. Tovar-Herrera OE, Batista-García RA, Sánchez-Carbente MR, *et al.* A novel expansin protein from the white-rot fungus Schizophyllum commune. *PLoS One.* 2015; 10(3):e0122296.
7. Guccione E, Hitchcock A, Hall SJ, *et al.* Reduction of fumarate, mesaconate and crotonate by Mfr, a novel oxygen-regulated periplasmic reductase in Campylobacter jejuni. *Environmental Microbiol.* 2009;12(3):576-591.
8. Blatt, WF, Robinson, SM, Bixler HJ. Membrane ultrafiltration: the diafiltration technique and its application to microsolute exchange and binding phenomena. *Anal Biochem.* 1968;26(1):151-173.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For additional information,
visit www.sartorius.com

Version 11 | 2021