SARTORIUS

Frequently Asked Questions

Laboratory Chromatography

1. Which modality and functional group should I choose for my molecule of interest?

Sartobind® Lab and Vivapure® units incorporate membranes as the chromatography matrix. These may be functionalized with various ion exchange or affinity ligands:

- Sulfonic acid (S) – a strong acidic cation exchanger
- Quarternary ammonium (Q) – a strong basic anion exchanger
- Diethylamine (D) – a weak basic anion exchanger
- Iminodiacetic acid (IDA) - for loading with the metal ion of your choice (Sartobind® Lab only)
- Protein A - for antibody affinity purification (Sartobind® Lab only)

For molecules you have already purified previously, we would recommend selecting membrane adsorber unit with the same modality and functional group you have used before. For new target molecules, scouting and optimizing the purification conditions should be a priority, and ideally would involve testing multiple modalities and chemistries. This should also take into consideration the molecule type and properties (e.g. pI, net charge in the chosen purification buffer system, and the presence of any tags), and the purpose and mode of chromatography (i.e. capture for polishing or purification, or flow through for contaminant removal).

2. How should I store Sartorius lab purification products and what are their respective shelf lives?

Units with IEX and IDA chemistries can be stored in a clean, dry place, away from direct sunlight, at room temperature. The shelf lives for these products are 5 years (Sartobind® IEX Lab), 4 years (Sartobind® IDA Lab) or 3 years (Vivapure® IEX). Sartobind® Rapid A Lab units can also be stored at room temperature and have a shelf life of 1 year.

3. What are the bed volumes in Sartobind® Lab and Vivapure® units?

Sartobind® Lab units are available in bed volumes up to 2.8 mL. Sartobind® Q | S Lab are offered with a choice of 0.41, 2.1 or 2.8 mL bed volumes, while Sartobind® D | IDA Lab each have a 2.1 mL bed volume and Sartobind® Rapid A Lab, a 0.5 mL bed volume.
Vivapure® Mini and Maxi have membrane areas of 7.48 and 84.4 cm^2, which are equivalent to bed volumes of 0.24 and 2.7 mL, respectively.

4. Can the lab purification units be sanitized?

The IEX units may be sanitized by washing once with 70% ethanol, 1 M HCl or 1 M NaOH, while 0.2 M NaOH is recommended for sanitizing Rapid A units. Residual sanitization solution can be removed by washing the units to the starting conditions with loading buffer at its usual working concentration for purification. If sterility is essential, the sanitization process should be validated. Due to the housing materials used, these units are **not** suitable for sterilization by autoclaving.

5. Do Sartobind® Lab and Vivapure® units need to be equilibrated before use?

Especially in capture applications, we recommend equilibration with a buffer solution similar in composition to the sample. With Sartobind® Lab units, this is achieved by flushing with up to 10-20 mL of loading buffer, while Vivapure® units can simply be filled with loading buffer followed by a rapid, single spin.

6. Do my purification buffers need to be degassed?

Unlike with conventional resin-based chromatography, there is no possibility of air bubbles disrupting the membrane matrix. Therefore,degassing is not required and this preparative step can be eliminated from your purification process.

7. Which buffer should I use for my application?

It is important to select a buffer which will not adversely affect the interaction of your molecule with the membrane matrix. For example, buffer agents which carry a charge opposite to that of the functional group on an ion exchange membrane adsorber may reduce the binding capacity. Similarly, high salt concentrations or extremes of pH also decrease binding capacities. Therefore, for optimal capture of the target molecule, it is preferable to use loading buffers comprising agents with the same charge as the membrane adsorber, and which have low ionic strength.

Subsequently, increasing the salt concentration or adjusting the pH of the buffer is useful during washing and elution steps. Using step or gradient elution with gradually changing salt content or pH may also help to increase the purity of the target molecule before effecting its release from the membrane.

In some cases, it may also be preferable to avoid using aromatic reagents in the purification buffers. These can interfere with subsequent spectrophotometric analyses of the purified target molecule - especially where it might not be possible to establish a reliable baseline absorbance spectrum. However, if their use cannot be avoided (e.g. imidazole in immobilized metal affinity purification), the concentration of these reagents can be reduced or they can be removed completely after purification, by diafiltration with Vivaspin® centrifugal concentrators.

8. What is the chemical compatibility of Sartobind® Lab and Vivapure® units?

It depends on the modality and functional group but in general, these units are resistant to most common chromatography reagents. For the ion exchange membrane adsorbers, they are typically stable against 1 M sodium hydroxide, 8 M urea, 6-8 M guanidine hydrochloride and 500 mM imidazole, as well as a wide range of organic solvents, detergents and reducing agents. For further information, please refer to the instructions for use for your chosen product.

9. How should I prepare my sample for purification?

For optimal purification, we recommend ensuring that your sample has a similar composition to the buffer used for equilibration and loading. This can be achieved simply by diafiltration with Vivaspin® centrifugal concentrators, simple dilution, or dialysis. The salt concentration of the sample should typically be ≤25 mM. To prevent membrane blocking, it is also recommended to remove particulates using a pre-filter such as, Vivaclear or Minisart® filters. Thanks to the Luer lock connectors, Minisart® syringe filters can easily be connected upstream of Sartobind® Lab units, enabling particle removal and purification in a single process

10. What is the direction of flow through Sartobind® Lab units?

The sample and buffers should be applied via the female Luer lock inlet, to flow through the device and emerge from the male Luer lock outlet. The flow direction is indicated by an arrow on each unit.

11. How should I orient Vivapure® IEX units for centrifugation?

For optimal performance, we recommend aligning the printed character on the purification insert of Vivapure® Mini units towards the centre of the rotor. The same recommendation applies when using Vivapure® Maxi in fixed angle rotors, although the use of a swing bucket rotor should be preferred for these units, since this ensures a uniform flow path of sample through the membrane adsorber.

12. What are the recommended RCF or flow rates?

Optimal performance is obtained when centrifuging at 500 or 2,000 *g* for Vivapure® Mini or Maxi units, respectively. The recommended flow rate for Sartobind® Lab units depends on the modality: an impressive 5 - 30 MV/min for ion exchange and metal affinity units or up to 50 MV/min for Rapid A.

13. Can Vivapure® IEX units run dry?

No – even through centrifugal operation, the membranes remain hydrated, so there is no risk of sample loss or degradation through drying out between loading, washing and elution.

14. How should I elute my target molecule?

In ion exchange, elution is typically achieved by applying a buffer with increased ionic strength (up to 1 - 2 M), which weakens the interaction between the target molecule and the ion exchange ligand. Alternatively, increasing the pH (for a cation exchanger) or decreasing the pH (for an anion exchanger) is also an effective means of elution.

Low pH elution is also commonly used during Protein A affinity purification. Please note that it is recommended to neutralize the purified sample immediately after elution. This can be easily achieved by performing elution into a tube already containing a neutralizing buffer (e.g. 1 M Tris, pH 9).

For immobilized metal affinity purification, imidazole at concentrations up to 100 - 500 mM is typically used to effect elution of the his-tagged target molecule.

Performing elution in multiple steps of increasing ionic strength (e.g. in 100 – 300 mM steps) or pH can be used to fractionate or separate proteins which are loosely and tightly adsorbed to the membrane matrix, or help to increase purity of the molecule of interest.

15. What are the minimum eluate volumes?

The minimum elution volume will depend on strength of target molecule adsorption to the membrane adsorber and the elution buffer composition. However, the final purified sample could be as little as 50 µL or 2 mL from Vivapure® Mini or Maxi spin columns. If the entire binding capacity has been utilized and 100% of the target molecule has been captured and eluted, this corresponds to 10-fold concentration of the initial sample.

If you require a lower volume or higher sample concentration for subsequent processing or analysis, eluates can be rapidly concentrated using Vivaspin® or Vivacon® centrifugal ultrafilters.

16. How should I choose between Sartobind® Lab and Vivapure® units?

Sartobind® Lab units are available with a broader choice of modalities, so if you need to purify by affinity purification instead of ion exchange, these are the ideal choice. They also offer flexibility in operation - connect them to a syringe, pump or FPLC system, without the need for additional, expensive accessories or connectors. Finally, these units can be reused, with Sartobind® Q Lab having been shown to maintain binding capacities for over 100 cycles. Whether you choose single use or reuse can be determined by cost-benefit calculations, which take consumable and cleaning costs into consideration.

Alternatively, Vivapure® units are single use spin columns. This makes them ideal for screening and scouting, by testing multiple samples or buffer conditions in parallel.

17. How can I choose between the different bed heights on offer?

Your decision will depend on the yield of your target molecule (and therefore the overall binding capacity of the membrane adsorber in each unit) and the purpose of purification.

Larger bed heights are typically recommended for capture (bind and elute) applications, in which the high dynamic binding capacity for large molecules can be best utilized. In contrast, for rapid contaminant removal and when binding capacity is less of a concern (e.g. in flow through mode for polishing), the lower bed heights are an excellent choice.

Finally, if you will need to transition to Sartobind® process scale solutions in the future (e.g. for manufacturing), consider that these are offered with the 4 mm bed height which can also be found in Sartobind® IEX Lab units with 2.1 mL MV.

18. What are the binding capacities for each unit?

Vivapure® units are available for the purification of macromolecules from samples with an initial volume of 100 µL to 19 mL. For larger sample volumes, the loading step could be repeated several times, or Sartobind® Lab should be chosen. Sartobind® Lab also benefits from the possibility to connect multiple units in series, thereby increasing the binding capacity.

- Sartobind® IEX Lab and Vivapure® IEX: Up to 80 mg
- Sartobind® IDA Lab: Up to 7.5 mg
- Sartobind® Rapid A Lab: 17.5 mg or more

19. Are application guides available for my requirements?

With an extensive range of lab chromatography application guides, highlights and notes, there is sure to be a review or data to support your choice of the optimal Sartobind® Lab or Vivapure® unit. Follow the link below or speak with your Sartorius contact for more information.

20. Can I test the performance of these lab chromatography products?

Yes! Please get in touch with your local Sartorius sales contact, who will be happy to discuss your requirements and arrange a sample or trial discount.

Furthermore, if you are looking for a ligand that is not currently available from Sartorius, please let us know by emailing labfiltrationpm@sartorius.com.

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

U.K.
Sartorius UK Ltd.
Longmead Business Centre
Blenheim Road, Epsom
Surrey KT19 9QQ
Phone +44 1372 737159

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

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