1. Which functional groups are available in the Vivapure® IEX range and how do I choose the optimal chemistry for my target molecule?

The Vivapure® Mini and Maxi devices utilize Sartobind® membrane adsorbers. These comprise a reinforced regenerated cellulose membrane, which is functionalized with one of three ion exchange chemistries:

- Sulfonic acid (S) – a strong acidic cation exchanger
- Quaternary ammonium (Q) – a strong basic anion exchanger
- Diethylamine (D) – a weak basic anion exchanger

To select the optimum chemistry, you should consider the calculated or theoretical isoelectric point (pI) of your target molecule and ensure that the pH of the sample loading buffer is at least 0.5 – 2 units above or below this value. If the sample buffer will have a pH below the pI, the target molecule will be positively charged and bind to a cation exchanger (Vivapure® S). At a pH above the pI, the target molecule will be negatively charged and bind to an anion exchanger (Vivapure® Q or D).

In cases where you have already purified a target molecule by ion exchange chromatography before, we would recommend selecting a Vivapure® spin column with the same functional group. For new target molecules, scouting and optimizing the purification conditions should be a priority, and ideally would involve testing multiple chemistries.

2. How should I store Vivapure® IEX devices and what is there a finite shelf life?

There is no need to refrigerate Vivapure® spin columns – they can simply be stored at room temperature. The shelf life is three years from the date of manufacture.

3. What is the bed volume of the ion exchange membrane in Vivapure® devices?

Vivapure® Mini and Maxi have membrane areas of 7.48 and 84.4 cm², which are equivalent to bed volumes of 0.24 and 2.7 mL, respectively.
4. Can Vivapure® devices be sterilized?

The spin columns may be sanitized by washing once with 70% ethanol or 1 M NaOH, removing residual sanitization solution by washing with 10x concentrated loading buffer, and then equilibrating the devices to the starting conditions with loading buffer at its usual working concentration. If sterility is essential, the sanitization process should be validated. Due to the housing materials used, Vivapure® devices are not suitable for sterilization by autoclaving.

5. Do Vivapure® IEX devices need to be equilibrated before use?

For optimal capture of the target molecule, we recommend equilibrating the device with an appropriate buffer solution, which is similar in composition to the sample buffer. This process can be achieved with 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 layers in Vivapure® devices. Therefore, there is no need to degas buffers prior to purification.

7. Which buffer should I use for my application?

It is important to select a buffer which will not adversely affect the ion exchange interaction. For example, buffer agents, which carry a charge opposite to that of the functional group on the membrane adsorber may reduce the binding capacity of the Vivapure® device. Similarly, high salt concentrations can also decrease binding capacities. Therefore, for optimal capture of the target molecule, it is preferable to use binding 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. This will help to increase the purity of the target molecule and effect 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, the concentration of these reagents can be adjusted or they can be be removed after purification, by diafiltration with Vivaspin® centrifugal concentrators.

8. What is the chemical compatibility like for Vivapure® IEX devices?

Vivapure® Mini and Maxi spin columns are resistant to most common chromatography reagents. They are stable against 1 M sodium hydroxide, 8 M urea, 6 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.
9. How should I prepare my sample for purification with Vivapure® devices?

For optimal purification, we recommend ensuring that your sample has a similar composition to the buffer used for equilibration of the Vivapure® device. 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 pre-filter samples with, for example, Vivaclear® centrifugal filters or Minisart® syringe filters.

10. How should I orient Vivapure® IEX devices in the centrifuge?

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

11. What is the recommended RCF for Vivapure® IEX spin columns?

Optimal performance is obtained when centrifuging at 500 or 2,000 g for Vivapure® Mini or Maxi devices, respectively.

12. Can Vivapure® IEX devices 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.

13. How should I elute my target molecule?

Typically, elution is 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.

Elution may also be performed in multiple steps of increasing ionic strength (e.g. in 100 - 300 mM steps) or pH to fractionate or separate proteins which are loosely and tightly adsorbed to the ion exchange matrix.

14. What is the minimum elution volume for Vivapure® IEX spin columns?

The minimum volume for elution from Vivapure® Mini devices is 50 µL. Vivapure® Maxi spin columns have a minimum elution volume of 2 mL.
15. What is the maximum concentration factor I can achieve with Vivapure® IEX devices?

When eluting samples in the minimum elution volume, it is possible to recover a sample of the target molecule which is approximately 10x more concentrated than in the original sample. This assumption is based upon the entire device sample capacity being utilized and 100% of the target molecule being captured and eluted.

Should the purified molecule need to be concentrated further after purification, Sartorius offers the most comprehensive range of lab ultrafiltration devices. Vivaspin® and Vivacon® centrifugal ultrafilters are ideal for handling the low eluate volumes from Vivapure® devices, typically enabling concentration factors more than 100-fold.

16. What are the sample capacities for each device?

Vivapure® devices are available for the purification of macromolecules from samples with an initial volume from 100 µL to 19 mL. Depending on the sample, it is possible to purify from a larger initial volume by repeating the loading step multiple times.

- **Vivapure® Mini:** Up to 400 µL
- **Vivapure® Maxi:** Up to 19 mL

17. Are application guides available for my requirements?

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

[Learn More]