Instructions for Use

Vivapure® Ion Exchange

Vivapure® Mini & Maxi Spin Columns

85030-513-22

SARTORIUS

Introduction

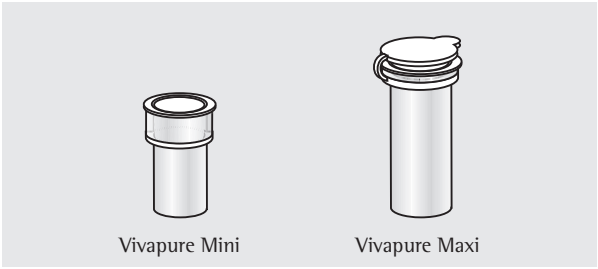
Storage conditions|shelf life
Store the Vivapure spin columns at room temperature. They can also be refrigerated at 4°C. These devices should be used within 3 years of purchase.

Vivapure Ion Exchange Spin Columns Introduction
With Vivapure ion exchange spin columns, separation is accomplished on the basis of the selective, reversible adsorption of charged molecules to an ion exchange group of the opposite charge. This is achieved in a 2 ml microcentrifuge tube for the Vivapure Mini spin column and in a 50 ml centrifuge tube for the Vivapure Maxi spin column.

Vivapure IEX replaces time-consuming, tedious and expensive chromatographic methods for many protein applications. The rapid purification protocol based on Membrane Adsorbers allows the parallel separation of proteins with high yield in less than 20 minutes.

Ion exchange chromatography is among the most widely used methods for fractionation and purification of biological substances.

The purification protocols with Vivapure IEX involve only a few steps, making the isolation of your target protein from samples almost as simple as filtration. The protein purified using the Vivapure spin column may be used in many downstream applications e.g. sample fractionation for 1D and 2D SDS-PAGE, X-ray crystallization, NMR spectroscopy and related applications.



Vivapure Mini Vivapure Maxi

Vivapure ion exchange spin columns are available as

Mini H	(0.4 ml volume – high binding capacity)
Maxi H	(19 ml volume – high binding capacity)

Three types of ion exchangers are available for separating charged biomolecules

Functional groups

Sulphonic acid (S)	$R\text{-}CH_2\text{-}SO_3^-Na^+$	Strong acidic cation exchanger
Quaternary ammonium (Q)	$R\text{-}CH_2\text{-}N^+\text{-}(CH_3)_3Cl^-$	Strong basic anion exchanger
Diethylamine (D)	$R\text{-}CH_2\text{-}NH^+\text{-}(CH_2H_5)_2$	Weak basic anion exchanger

	Working pH	**Approx. pKa of ionic group**
Vivapure IEX D	pH 4–10	9.5
Vivapure IEX Q	pH 2–12	11
Vivapure IEX S	pH 2–12	1

Vivapure IEX	**Protein binding capacity* [mg] spin columns**	**Max. loading volume per centrifuge run (ml) using swing-out rotor**	**Max. loading volume per centrifuge run (ml) using fixed-angle rotor**
Vivapure IEX Mini H	4	0.4	0.4
Vivapure IEX Maxi H	60–80	19	10.5

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

Technical Specifications

Membrane and device specifications

Supporting matrix	Stabilized regenerated cellulose
Nominal pore size	3–5 µm (Large pore size prevents gel filtration effects and minimizes non-specific adsorption)
Thickness	230–320 µm
Amount of ionic groups (µEquivalents/ml)	145–218 µEquivalents/ml for monovalent ions (D, Q & S)

	Bed Volume	Membrane Area (cm^2)
Vivapure Mini H	240 µl	7.48
Vivapure Maxi H	2700 µl	84.40

Compressibility
Deformation of resin-based gels can be a problem as channels between particles are closed down. The Vivapure ion exchange membranes resist any deformation or volume change induced by the changing osmotic pressure resulting from pH and ionic strength variations.

Internal Surface Area
A 1 cm^2 piece of membrane has a total (internal and external) surface area of approximately 100 cm^2.

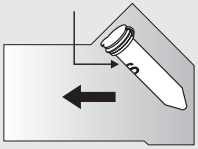
Required Equipment

Vivapure Mini spin columns
Any microcentrifuge that will accommodate 2.0 ml micro-centrifuge tubes and can spin samples at speeds of 2000 x g. You must consider the orientation of the Vivapure Mini spin columns in a fixed-angle rotor.

Vivapure Maxi spin columns
Ideally, a bench-top centrifuge with swingout rotor that can accommodate 50 ml centrifuge tubes and can spin samples at 500 x g. With a fixed-angle rotor you must consider the orientation of the Vivapure Maxi and lower maximum volume permitted.

For both Mini and Maxi spin columns
Syringe filters; 0.8 µm for clarification of visible particles, 0.45 µm for fine filtration. (product no: 16592K, 0.8 µm – pack of 50; 16555K, 0.45 µm – pack of 50).

Orientation of Vivapure spin columns in a fixed-angle rotor



To achieve optimal performance of Vivapure spin columns in a fixed-angle rotor, we recommend aligning the printed character (e.g. Q, D or S) on the insert towards the centre of the rotor for the binding, washing and elution steps. This measure guarantees even liquid flow through the membrane during all chromatography steps.

The membrane adsorber
The Vivapure IEX range uses the same patented membrane technology found in the process-scale Sartobind® and SingleSep® family supplied by Sartorius Stedim Biotech. Generally, Vivapure ion exchange membranes facilitate and speed up all ion exchange applications in which step gradient elution is conducted.

Operating Vivapure IEX Mini and Maxi spin columns
In order to prevent clogging of the spin columns, we recommend that you pre-filter your samples with a syringe filter (e.g. Minisart, 0.45 µm pore size, product no. 16555K).

Effective ion exchange chromatography demands careful consideration of pH and ionic strength of the loading buffer. You may therefore need to reduce the ionic strength of your sample by simple dilution, dialysis or ultrafiltration before loading it on to the Vivapure IEX spin column. It is best to dilute your sample in loading buffer of concentration equal to or less than 25 mM salt.

1-2-3 protocol
For spin speed and time, see table 1 for Mini and Maxi spin columns.

- Equilibrate spin column with equilibration buffer
- Load sample in as many runs as necessary
- Wash with equilibration buffer
- Elute sample in as many steps as necessary (with increasing salt concentrations).

1-2-3 protocol: For binding proteins to the Vivapure IEX Mini and Vivapure IEX Maxi spin columns

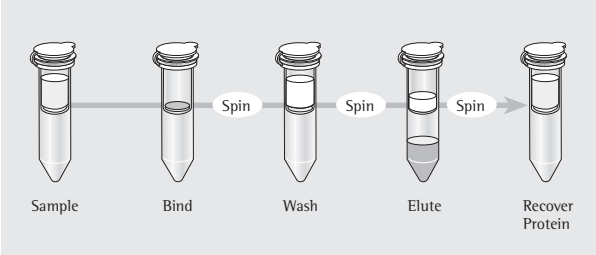


Table 1: Spin speeds and recommended buffer volumes

Device	Vivapure IEX Mini H [S, Q, D]	Vivapure IEX Maxi [S, Q, D]
Centrifugation speed	2000 x g	500 x g
Centrifugation time	5 min.	5 min.
Equilibration Buffer volume	0.4 ml	5 ml
Sample volume	0.4 ml; load sample in as many 0.4 ml runs as necessary	19 ml – swing-out, 10.5 ml fixed angle; load sample in as many 19 ml/10.5 ml runs as necessary
Wash Buffer volume	0.4 ml	10 ml
Recommended Elution Buffer volume	0.4 ml	10 ml
Minimum Elution Buffer volume	0.05 ml	2 ml

Ion Exchange Guidelines

Choosing the optimal IEX group
The principal decisions for choosing an ion exchange adsorbent are as follows:

(1) the ion exchange charge, cation (negative) or anion (positive),

(2) the nature of the group responsible for that charge, (weak or strong ion exchanger group),

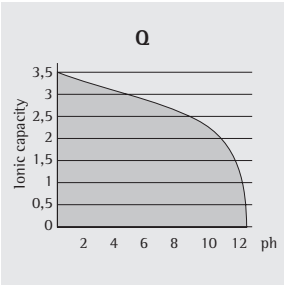
(3) the capacity and volume of the spin column.

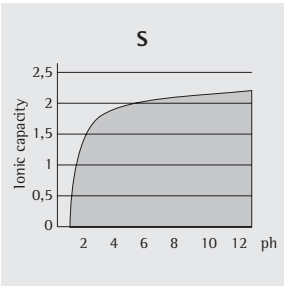
Ion exchangers are sub-divided into strong and weak exchangers. Strong ion exchange groups are completely ionized and exist as charged forms at nearly all pH values, whereas weak exchangers possess groups which are ionized only over a narrow pH range.

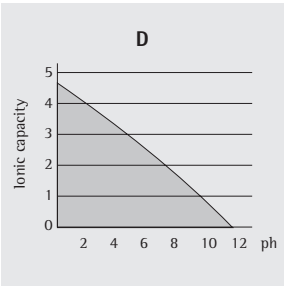
For example, the strong cation (S) and anion exchanger (Q) retain their charge over a larger pH range than the weaker anion exchanger (D).

The weak anion exchanger Vivapure D should be used below its specific pKa value of approximately pH 9.5.

Ionic capacity of the Vivapure IEX spin columns
The following graphs highlight the ionic capacities of the four ion exchange membranes as a function of pH.







The iso-electric point
As proteins are usually handled in buffered media, we are interested in their iso-electric points (pl).

The pl value of a given protein is defined as the pH at which the protein's net charge is zero. The pl value is an indication of a protein's net acidic or basic character which is also reflected in the net charge that it bears in solution at pH 7.

Fairly reliable values have been obtained for many proteins in media of comparatively low ionic strength and it has been shown that the pl values of most proteins are generally less than 7, although they cover a very wide range from pl 1.1 for pepsin to pl 12 for some protamines.

Note, however, that the iso-electric point of a protein is also the pH at which it is least soluble and most easily precipitated. This is probably due to mutual interaction between charged groups of neighbouring molecules: the number of opposing charges will be greatest at the iso-electric point.

Purification example
A protein with pl 9 ...
- will be positively charged at pH 7
- will bind to S membrane
- can be eluted with increasing salt concentration or by raising the buffer pH over 9.

A protein with pl 4 ...
- will be negatively charged at pH 7
- bind to Q membrane
- can be eluted with increasing salt concentration or by lowering the buffer pH under 4.

Vivapure IEX Chemical Compatibility

The compatibility of chemicals to the Vivapure IEX spin columns is highlighted in the following table. The ion exchange membranes have high chemical stability to organic solvents. In common to all other ion exchange resins, they are, however, susceptible to oxidative agents such as hydrogen peroxide.

Vivapure IEX chemical compatibility

Agent	Concentration	Device type		
		S	Q	D
Alcohols				
Methanol	98%	■	■	□
Methanol	60%	■	■	■
Ethanol	99%	■	■	■
n-propanol	100%	■	■	□
n-propanol	60%	■	■	■
Isopropanol	100%	■	■	□
Isopropanol	60%	■	■	■
Butan-2-ol	99%	■	■	■
Glycerol	100%	■	■	■
Ethylene glycol	20%	■	■	■
Polyethylene glycol	20%	■	■	■
Ketones				
Acetone	100%	■	■	■
Methylethyl ketone	100%	■	■	■
Acids				
Acetic acid	1.0 M	■	■	■
Formic acid	25%*	■	■	■
Hydrochloric acid	1.0 M	■	■	■
Sulphuric acid	1.0 M	■	■	■
Trifluoroacetic acid	2 M	■	■	■
Bases				
Ammonium hydroxide	28%	■	■	□
Ammonium hydroxide	10%	■	■	■
Sodium hydroxide	1.0 M	■	■	■
Detergents				
n-octyl b-D-glucopyranoside	2.0%	■	■	■
SDS	2.0%	■	○	○
Triton X-100	2.0%	■	■	■
Tween 20	2.0%	■	■	■

Agent	Concentration	Device type		
		S	Q	D
Oxidising agents				
Hydrogen peroxide	0.9 M	○	○	○
Sodium hypochlorite	200 ppm	○	○	○
Culture media				
DMEM	norm	■	■	■
RPMI-1640	norm	■	■	■
Solvents containing nitrogen				
Guanidine HCl	6 M	■	■	■
Urea	8 M	■	■	■
Other chemicals				
B-mercaptoethanol	100 mM	■	■	■
Chloroform	100%	■	■	■
Dichloromethane	100%	■	■	■
Dimethylformamide	100%	■	■	□
Dimethylformamide	50%	■	■	■
Dimethylsulfoxide	100%	■	■	■
Dithiothreitol	100 mM	■	■	■
EDTA (sodium salt)	5%	■	■	□
Imidazole	500 mM	■	■	■
Sodium chloride	5 M	■	■	■

* pH 1.0 solution

■ Compatible
□ Limited compatibility
○ Not compatible
Binding capacity may be affected.

Ordering Information

Cat Number	Vivapure IEX Mini	Spin Columns	Centrifuge Tubes
VS-IX01SQ16	Vivapure Mini S & Q (8 S & Q each)	16	32
VS-IX01DH24	Vivapure D Mini H	24	48
VS-IX01QH24	Vivapure Q Mini H	24	48
VS-IX01SH24	Vivapure S Mini H	24	48

Cat Number	Vivapure IEX Maxi	Spin Columns	Centrifuge Tubes
VS-IX20DH08	Vivapure D Maxi H	8	16
VS-IX20QH08	Vivapure Q Maxi H	8	16
VS-IX20SH08	Vivapure S Maxi H	8	16

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Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

Last updated:
08 | 2021

AM | Publication No.: SLU6101-e210806