HiFliQ S-type FPLC Columns

User Guide

Protein Ark HiFliQ columns pre-packed and ready to use with sulphonic acid propyl linked (S) coupled Agarose resin for rapid Ion Exchange (IEX) purification of positively charged proteins under native conditions.

Available in 1 ml and 5 ml HiFliQ column sizes with high ligand density and high binding capacity. Compatible with all common chromatography HPLC and FPLC instruments (including ÄKTA™ FPLC's), and low pressure pumps and syringes using an appropriate adaptor.

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Features of the HiFliQ S-type FPLC columns:

- Fast and reliable affinity purification.
- Pre-packed 1 ml and 5 ml columns with sulphonic acid propyl linked Agarose resin.
- Highly stable 6% cross linked Agarose with coupled sulphonic acid propyl ligand provides high buffer stability and broad compatibility with a very wide range of detergents, reducing agents and other additives.
- High binding capacity for positively charged proteins.
- Simple bind-wash-elute procedure.
- Biocompatible polypropylene casing.
- Universal 10.32 (1/16'') UNF threads (Inlet Female/Outlet Male) compatible with all common chromatography instruments (including ÄKTA™ FPLC's).
- Compatible with low pressure pumps (requires a 1/16'' male connector) and syringes (requires a Luer Female – 1/16'' male connector).
- Connect in series for increased capacity.

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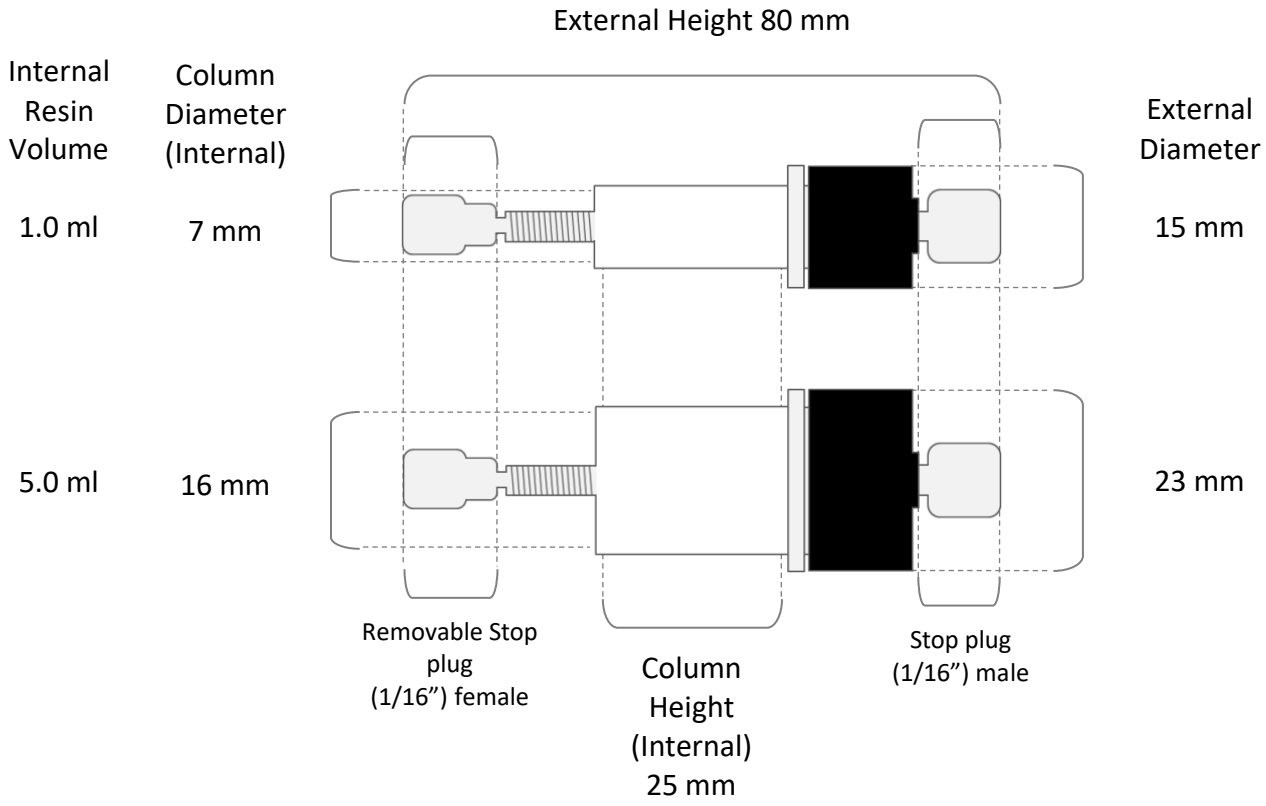
Specifications:

Item:	HiFliQ1 S-type IEX	HiFliQ5 S-type IEX
Column Volume:	1 ml	5 ml
Resin:	Sulphonic acid propyl linked Agarose	Sulphonic acid propyl linked Agarose
Typical Binding Capacity:	50-70 mg	250-300 mg
Base Matrix:	6% cross-linked Agarose	6% cross-linked Agarose
Mean Bead Size:	90 µm	90 µm
Recommended flow rate:	1 ml/min	1-6 ml/min
Max. operating pressure:	0.5 MPa (72 psi)	0.5 MPa (72 psi)
External Dimensions:	15 mm D. x 80 mm H.	23 mm D. x 80 mm H.
Column Construction:	Polypropylene	Polypropylene
Inlet Port:	10-32 (1/16") Female	10-32 (1/16") Female
Outlet Port:	10-32 (1/16") Male	10-32 (1/16") Male
Storage (2-30°C):	20% ethanol 0.2M Sodium Acetate	20% ethanol 0.2M Sodium Acetate

HiFliQ S-type FPLC column schematic:



NOTE:	HiFliQ FPLC columns are supplied with a moulded removable (1/16") female stop plug attached to the (1/16") male outlet port. Remove prior to use, reverse and use to seal the column for storage.
NOTE:	HiFliQ FPLC columns cannot be opened or repacked.

Chemical compatibility:

Buffer compatibility	
Standard buffers:	Common aqueous buffers and salts
pH range:	2.0-14.0
Chelating agents	
EDTA:	1 mM
Sulfhydril reagents	
β-mercaptoethanol:	5 mM
DTT:	10 mM
Denaturants	
Urea:	8 M
Guanidinium hydrochloride:	6 M
Detergents	
DDM (n-Dodecyl-β-Dmaltoside):	0.1%
OG (n-Octyl-β-Dglucopyranoside):	5%
Triton® X-100:	Up to 2 %
Tween® 20:	Up to 2%
NP-40:	0.2%
SDS:	Up to 0.03% (w/v)
Cetyltrimethylammonium bromide (CTAB):	UP to 1%
C12E8:	0.05%
Brij 35:	0.1%
Cholate:	5%
Deoxycholate:	2%
CHAPS :	1%
Other additives	
NaCl:	1 M
NaOH:	0.5-1.0 M
HCl:	0.1 M
Ethanol:	70% (v/v)
Isopropanol:	30%

Principles of Ion Exchange Chromatography:

Ion Exchange Chromatography (IEX) is one of the most common and versatile method for fractionating biological substances. Proteins and peptides with small differences in charge can be separated. The quaternary ammonium group is a strong cation (positively charged resin) useful for high-resolution separations and purification of negatively charged proteins.

Sulphonic acid propyl linked resin:

Sulphonic acid propyl group immobilised onto 6% cross-linked agarose beads for clear separation of negatively charged proteins.

Application drivers for S-type IEX chromatography:

Purification of recombinant proteins for raising antibodies.
Purification of recombinant proteins for activity and/or structural studies.

General considerations for selecting optimal binding conditions for the S resin:

The key parameters affecting the binding of positively charged proteins to the IEX resin are the flow rate over the column and buffer conditions. Binding is most effective when equilibration buffer has a pH of at least 0.5 units below the iso-electric point (pI) of the target protein. The ionic strength of the equilibration buffer should also be low, with preferably no or minimal salt present (<50mM).

If the binding efficiency is found to be poor and the lysis buffer differs significantly from the pre-equilibration buffer, it is recommended that the lysate be dialysed, titrated with a concentrated stock solution, or buffer exchanged using an ultrafiltration device with a more appropriate pre-equilibration buffer.

It is imperative that the lysate/clarified sample is completely clear prior to loading on the column as any particulate matter (e.g. cell debris) may clog-up the resin resulting in an increased back pressure and reduced flow rates. This will significantly increases the binding, washing and elution times and affects the final purity of the eluted target protein. It is recommended that the cleared lysate/clarified sample is filtered using a 0.22μm filter prior to loading onto the S column. Ideally samples should be processed rapidly and, if the need arises, the protein purified at 4°C. It is also recommended that the number of freeze/thaw cycles be minimised during storage to reduce the amount of aggregation/precipitation of the proteins.

Optimal buffer conditions for binding the target molecule to a resin are critical for successful purification of the protein. If the binding conditions are not optimal with respect to pH, salt concentration, detergent ...etc, purification can be adversely affected.

Operating Procedure

1. Equilibrate the column with up to 5-10 CV (Column Volumes) of equilibration buffer
2. Apply the clarified / filtered sample onto the equilibrated column. Recommended residence time of 3 minutes (or greater)
3. Remove any non-bound material in the column with up to 5-10 CV of equilibration buffer, or until the UV trace returns to baseline.
4. Elute the bound protein by using a salt gradient from 0 - 1M over 20-40 CV.
5. If a clean in place (CIP) is required, use 5-10 CV of 1.0 M NaOH. A contact time of 1 hour will normally suffice to ensure destruction of viable organisms, although up to 5 hours contact time may be required. No less than 5 column volumes are recommended.
6. Re-equilibrate column with up to 10 CV of equilibration buffer (to remove sodium hydroxide) until the pH and conductivity of the column eluate is equal to that of the buffer entering the column before storage or re-use
7. If the column is to be stored for future use, place the column into the storage solution (20% ethanol recommended) and store at 2 – 8°C

Column washing and regeneration conditions:

Protein Ark HiFliQ S FPLC columns should be carefully washed with PBS (wash buffer) and stored in 20% ethanol after each run. If the column shows increased back pressure or loss of binding capacity then this may be due to the accumulation of precipitated, denatured, or non-specifically bound substances and proteins. This protocol delineates washing and regeneration procedures for the 1 and 5 ml HiFliQ S FPLC column to remove these substances and proteins. All volumes are given in column bed volume (CV).

Example: Buffers washing and regeneration.
Wash buffer:
Buffer + 1-2M NaCl
1M NaOH
10-100 mM Sodium phosphate, 2.7 mM potassium chloride, 0.137 M NaCl, pH 7.4 (PBS)
Storage buffer:
20% Ethanol, 0.2M Sodium Acetate

Wash and regeneration procedure 1: removal of precipitated and denatured substances and proteins.

1. Connect the HiFliQ S column to an FPLC/syringe/pump and wash with 10 CV's of '2.0M NaCl'.

2. Wash the column with 10 CV's 'dH_2O'.

3. Immediately wash the column with 10 CV's of '1.0M NaOH'.

4. Wash the column with 10 CV's of 'equilibration buffer' and for long term storage, wash with 10 CV's of 20% Ethanol and store at 4°C.

NOTE: The column is now ready for re-use if required.

NOTE: If wash procedure 1 does not fully resolve the problem then repeat.

Storage conditions:

Item:	HiFliQ1-S	HiFliQ5-S
Shipping:	20 % Ethanol, 0.2M Sodium Acetate at room temperature	20 % Ethanol, 0.2M Sodium Acetate at room temperature
Short-term storage:	Equilibration buffer	Equilibration buffer
Long-term storage:	20% Ethanol, 0.2M Sodium Acetate at 4°C	20% Ethanol, 0.2M Sodium Acetate at 4°C

NOTE: Do not freeze or store the column in buffer or water for long periods. Each column is stored in 20 % ethanol and reusable for up to 2 years from the date of resin manufacture. Please see the label for the recorded expiry date.

Performance Data:

Purification of Protein X from *E.coli* lysate

Sample	Column	Instrument	Flow Rate	Binding Buffer	Elution Buffer
E.coli lysate	1ml HiFliQ-S (a) 5ml HiFliQ-S (b)	FPLC	1ml/min	50mM NaOAc pH5.0	50mM NaOAc 1M NaCl pH5.0

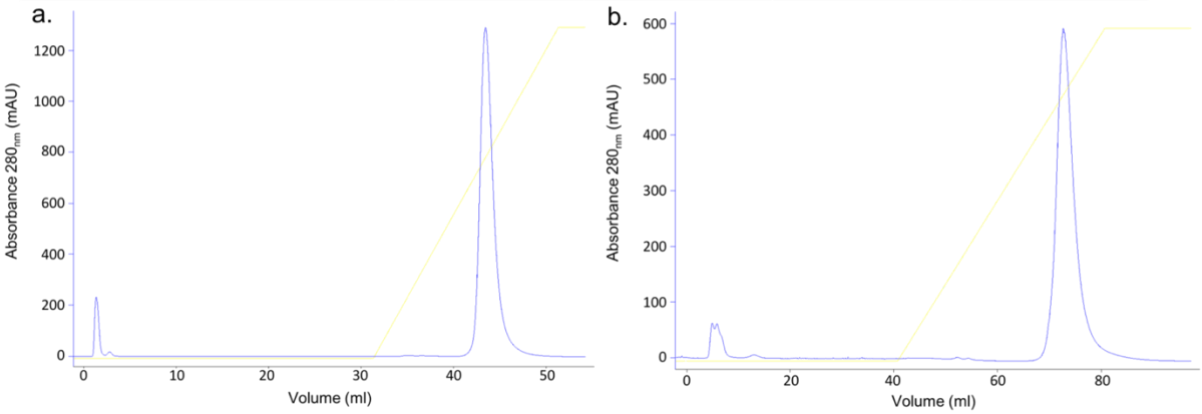


Figure 1. 0.1ml of *E.coli* lysate was treated with 10mg/ml of BSA and applied to 1ml (Fig. 1a) and 5ml (Fig. 1b) HiFliQ S-type FPLC columns to purify Protein X. Samples were loaded onto columns coated with binding buffer, and eluted (elution gradient in yellow) over 20 CVs from 0-1M NaCl. Protein X was eluted at an elution gradient of ~0.6M NaCl (1ml HiFliQ) and ~0.8M NaCl (5ml HiFliQ).

FAQ:

1. *What is the shelf-life of the HiFliQ S FLPC Column?*
   The resin is guaranteed for 2 years after the date of manufacture provided they are stored at 2-8°C.

2. *Do I need to filter the buffers prepared in my laboratory?*
   It is good laboratory practice to filter all buffers.

3. *How should I prepare my sample for IEX chromatography?*
   Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. We recommend that all samples are filtered to at least 0.45 µm (preferably 0.20 µm) pore size. High viscosity is mostly attributed to contaminating DNA or RNA. The intrinsic viscosity of a lysate can be reduced by either drawing it through a syringe needle several times or by adding appropriate amounts of DNase and/or RNase (5-10 µg/ml) to the lysis buffer and incubating the mix on ice for 15 mins.

4. *How can I regenerate the HiFliQ S FPLC comulm?*
   We recommend that you wash the column with PBS and store in 20% ethanol between each run. If column performance and binding capacity becomes reduced then we recommend washing with harsher conditions. See 'Operating Procedures' for further details.

5. *Should I be concerned if the column partially dries out during the chromatographic steps?*
   The resin is robust although we recommend flushing out as much air as possible from the column before continuing. Partially dried resin rehydrates rapidly however the performance of the column (binding capacity and running pressure) may be affected.

6. *Can I load purified protein immediately on to an SDS-gel?*
   Proteins purified from the HiFliQ S FPLC column under the recommended conditions can be loaded on to an SDS-polyacrylamide gel.

7. *Under what circumstances can I re-use the column?*
   The HiFliQ S FPLC columns are designed for re-use. We recommend regular washing and cleaning between purifications in order to maintain performance. Should you observe a slowdown in flow rate or increase in back pressure then we recommended washing and regenerating the column prior to further use. See 'Column washing and regeneration conditions' for further details.

Troubleshooting assistance:

Bubbles or cracks appear in the resin bed:

- The resin has been stored at a cool temperature and then rapidly warmed up. The resin should be warmed slowly to room temperature before use.

The sample does not flow easily through the column:

- The resin is clogged with particulates. Pre-filter the sample just before loading it on to the column.
- If the column is not stored at 2-8°C, or it has been used more than once and stored in the absence of a bacteriostat, microbial growth may restrict flow through the column.

No binding or elution of the target protein is observed from the column:

- Extensive sonication can denature the protein preventing the protein from binding to the column.
- Check the pH of the Equilibration buffer. It is recommended to use an equilibration buffer of at least 0.5 units above the pI (Isoelectric point) of the target protein.
- Test the binding conditions and HiFliQ S FPLC column are working correctly using just the expressed protein sample for bind-wash-elution.
- The retention time may not be sufficient for binding. Reduce the flow rate of the loading stage down to 0.2-0.5 ml/min (1 ml HiFliQ S column) or 1-3 ml/min (5 ml HiFliQ S column).
- The column may contain a build-up of precipitated, denatured, or hydrophobic substances and proteins which may impede binding. See 'Operating Procedures' for details of washing and regeneration protocols or use a new HiFliQ S FPLC column.

The recovery of target protein is low:

- Increase the elution time and volume of the 'Elution buffer'.

Poor resolution of the target protein:

- The sample volume or concentration may be too large for the capacity of the column. In this case, reduce the sample load or sample volume.
- The sample may need to be filtered carefully.

Glossary:

cleared lysate – the soluble cell extract after the cell debris and other particulates have been removed by centrifugation.

column bed volume (CV) - the total volume occupied by the chromatographic packed bed. It is also referred to as the column volume or CV.

DL-Dithiothreitol (DTT) – reducing agent used to break disulphide bonds.

expression vector – a cloning vector intended for the foreign gene to be expressed in the host organism.

freeze-thawing – a method that is sometimes used to break open cells by successive periods of slow freezing and thawing. Ice crystals are generated during the freezing stage, which disrupt the cells when they melt during thawing. The method, however, is slow and releases a limited amount of subcellular components.

immobilized - bound to a surface, usually through covalent linkages.

ion exchange chromatography - chromatographic separation based on different charge properties of macromolecules.

isoelectric point - the pH at which the protein has no net charge.

recombinant protein – a protein coded for by a cloned gene which has often been modified *to increase the expression of that protein or to alter the properties of the protein.*

tris(2-chloroethyl) phosphate (TCEP) – strong irreversible reducing agent used to break disulphide bonds.

truncate - terminate prematurely or to shorten by cutting.

Ordering information:

Product	Volume	Order Code
1 ml HiFliQ S FPLC column (1 x 1 ml)	1 x 1ml	HiFliQ1-S-1
1 ml HiFliQ S FPLC columns (5 x 1 ml)	5 x 1ml	HiFliQ1-S-5
5 ml HiFliQ S FPLC column (1 x 5 ml)	1 x 5 ml	HiFliQ5-S-1
5 ml HiFliQ S FPLC columns (5 x 5 ml)	5 x 5 ml	HiFliQ5-S-5

Technical support:

Contact the Protein Ark technical support and sales centre for assistance:

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