HiFliQ AAVX Long Bed FPLC Columns
IFU

2mL AAVX 7x50 HiFliQ Column
5mL AAVX 11x50 HiFliQ Column
10mL AAVX 16x50 HiFliQ Column
25mL AAVX 26x50 HiFliQ Column
50mL AAVX 26x100 HiFliQ Column
100mL AAVX 50x50 HiFliQ Column

General Information

Column body (i.e. the tube): made of acrylic. It has an appearance similar to a glass tube, i.e. clear and transparent. This material is compatible to most commonly used aqueous chemicals. ***WARNING: It isn't compatible with concentrated alcohols. 20% ethanolcan be used for storage purpose. Don't use any alcohols greater than 20% v/v.***

End plunger: made of PP with polyamide support mesh (15 □m). Its o-ring is of NBR.They are inert to most aqueous buffers.

Connection: 1/16" female thread in both sides, compatible to most chromatographysystems including AKTA

Blue End Cover: Made of POM

Operating pressure: column hardware rating is less than **5 bar** (or 0.5 MPa, or 70 psi).

Instruction of Use

Each packed column is sealed with a pressured syringe in the ***bottom*** end of the column.It is then placed in sealed plastic bag.

1. Cut the plastic bag to take out the packed column with care.
2. Follow the flow direction to clamp the column to a vertical stand.
3. Get the chromatography system ready for connection.
4. Unscrew the stop plug. Some liquid may come out.
5. Connect the column top to the inlet of the chromatography system.
6. Gently unhook the springs from the shaft top of the bottom syringe using balancedforce.
7. Twist with push-up force to unscrew the male-thread adaptor from the column top.Keep this storage syringe for later use.
8. Connect the bottom side of the column to the chromatography system.
9. Run at reduced flowrates to wash away the storage solution then run at normalflowrate until the column is equilibrated.

Storage after Use

In short term, seal both ends of the column with stop plugs if it isn't used.In longer

term, follow the instructions below to store the column.

1. Fill the column with suitable storage solution. Typically, it requires to pass at least 2CV storage solution.
2. Stop the pump.
3. Suck the storage solution to fully fill the storage syringe (including the male-threadand the tubing part). Leave the syringe upside-down and push out the air bubble. Adjust the liquid level inside the syringe to 3.5 ml to 4 ml (for 0.8 to 1.1 cm ID columns) or 11 to 12 ml (for 2.6 cm ID columns).
4. Carefully screw the male thread part of the syringe system to the outlet of thecolumn. Finger tight is enough.
5. Dis-connect the column top from the chromatography system.
6. Screw a 1/16" stop plug to seal the column top.
7. Use balanced force to hook the springs back the top shaft of the syringe.
8. Check and make sure no leakage.
9. Place the column in a cold room.

Resin Specification

Column Characteristic	**Description**
Resin Support Matrix	Cross-linked poly(styrene-divinylbenzene)
Resin Ligand	Single domain (VHH) antibody fragment
Binding Capacity	>1x10^{13} genome copies /ml of resin
Serotype Affinity	AAV1 through AAV9
Particle Size	50µm
Column Storage Buffer	20% Ethanol, 1M NaCl
Max Column Hardware Operating Pressure	0.5 MPa
Mechanical Resistance of Resin	10 MPa
Recommended Flow Rates	Max 4ml/min to remove 20% ethanol or introduce 20% ethanol as storage)
Storage Conditions	2-8˚C
Shipping Conditions	Ambient

Chromatography Conditions - Technical Guidelines

Guidelines for Equilibration and Binding Conditions:

Start with PBS (pH 7.0 to 7.5) as an initial buffer choice. Other neutral pH buffers like 10-50 mM sodium phosphate or Tris can also be used, however the pH must be in the range of pH 6-8. The addition of 0.1 – 0.2 M NaCl or KCl may prevent protein/protein interactions that can lead to nonspecific adsorption.

Guidelines for Wash Conditions:

Following the sample load, wash any unbound material from the column with the equilibration buffer. Usually an initial wash of 5-10 CV is enough to remove all unbound proteins from the column. If required, a secondary or intermediate wash step can be performed to increase impurity removal. Secondary/ intermediate wash options include:

- A high-salt wash with up to 1M NaCl
- Varying the pH
- Inclusion of additives (such as Tween™ 20 up to 0.05 (v/v)%
- Chaotropic salts (e.g. <0.2M $MgCl_2$)
- <20% ethanol (should avoid capsid damage)

*To avoid over-pressurising the column, avoid using buffers containing phosphates which can lead to precipitation.

Guidelines for Elution Conditions:

Note: Target molecules differ in their binding and elution behaviours. Therefore, we recommend determining the best elution conditions experimentally.

- A good starting point is a simple elution buffer such as 50-100 mM citric acid pH 3.0.
- Most target molecules will elute in a pH range of 2 to 3.
- Other buffer components such as acetate, glycine, hydrochloric acid or phosphate, that are effective at a low pH can also be used. For further optimisation of elution conditions look at using combinations of the above.
- Ensure a good pH transition by using an elution buffer strength that is greater than the equilibration buffer strength.
- Consider using a step elution (for a concentrated elution fraction) followed by a gradient for additional separation of similar product impurities.
- Do not underload the column.
- Following elution, immediately neutralize the eluted pool to prevent low pH denaturation.

Guidelines for Resin Cleaning and Storage:

Column Cleaning:

To ensure a long column performance liftetime, clean the column in place (CIP) after every sample run. Reverse the flow direction of the column to flush out any particulates and to clean the lower part of the column bed. Slowing the flow rate is also recommended to prolong exposure to the regeneration solution.

Note: Use cleaning solutions in the range of pH 2-12. This resin is acid stable and has limited caustic stability. Look at testing cleaning solutions in the following order:

- Lower pH Elution Buffer (pH 1.5-2.0)
- Lower pH Elution Buffer with added 1-2 M NaCl
- 0.1-0.5 M citric acid
- 0.5-1.0 M acetic acid
- 0.5 M phosphoric acid
- 6 M urea
- 2-6 M guanidine hydrochloride
- 20% ethanol or 20% isopropanol
- 10-25 mM NaOH

An example of typical cleaning process that can be used is:

- Strip column with 0.1 M phosphoric acid (pH 2.0)
- Clean step with 6 M guanidine hydrochloride

- Re-equilibrate with a neutral pH buffer such as PBS (pH 7.5) or store column in buffered ethanol

Resin Storage:

The bulk resin should be stored at 2 to 8°C. Do not freeze the resin. Store packed columns at 2 to 8°C (for long-term storage) or at room temperature for short-term, after cleaning. A recommended storage solution is 20% ethanol with 0.1 M sodium phosphate (pH 7.0).

Questions and answers:

1. What is the shelf-life of the HiFliQ AAVX 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. 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.

4. Under what circumstances can I re-use the column?
   The HiFliQ AAVX FPLC columns are designed for re-use. We recommend regular cleaning between purifications in order to maintain performance.

Ordering Information

Product Code	Description	Pack size
HiFliQ0.2-AAVX-1	0.2 ml AAVX 6x7 HiFliQ column, (1 x 0.2ml)	1
HiFliQ0.2-AAVX-5	0.2 ml AAVX 6x7 HiFliQ column, (5 x 0.2ml)	5
HiFliQ2-AAVX-1-LB	2ml AAVX 7x50 HiFliQ column, (1 x 2ml)	1
HiFliQ2-AAVX-5-LB	2ml AAVX 7x50 HiFliQ column, (5 x 2ml)	5
HiFliQ5-AAVX-1-LB	5ml AAVX 11x50 HiFliQ column, (1 x 5ml)	1
HiFliQ5-AAVX-5-LB	5ml AAVX 11x50 HiFliQ column, (5 x 5ml)	5
HiFliQ10-AAVX-1-LB	10ml AAVX 7x25 HiFliQ column, (1 x 10ml)	1
HiFliQ10-AAVX-5-LB	10ml AAVX 7x25 HiFliQ column, (5 x 10ml)	5
HiFliQ25-AAVX-1-LB	25ml AAVX 7x25 HiFliQ column, (1 x 25ml)	1
HiFliQ25-AAVX-5-LB	25ml AAVX 7x25 HiFliQ column, (5 x 25ml)	5
HiFliQ50-AAVX-1-LB	50ml AAVX 7x25 HiFliQ column, (1 x 50ml)	1
HiFliQ50-AAVX-5-LB	50ml AAVX 7x25 HiFliQ column, (5 x 50ml)	5
HiFliQ100-AAVX-1-LB	100ml AAVX 7x25 HiFliQ column, (1 x 100ml)	1
HiFliQ100-AAVX-5-LB	100ml AAVX 7x25 HiFliQ column, (5 x 100ml)	5

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