

## HiFliQ AAVX Short Bed FPLC Columns

### IFU

BioServUK's HiFliQ AAVX FPLC columns pre-packed and ready to use with AAVX resin for affinity purification of native and recombinant adeno-associated virus (AAV) subtypes.

The AAVX resin is the gold standard resin used for the purification of AAV particles.

BioServUK's pre-packed lab scale AAVX columns offer high specificity, capacity and maximises yield by reducing process steps.

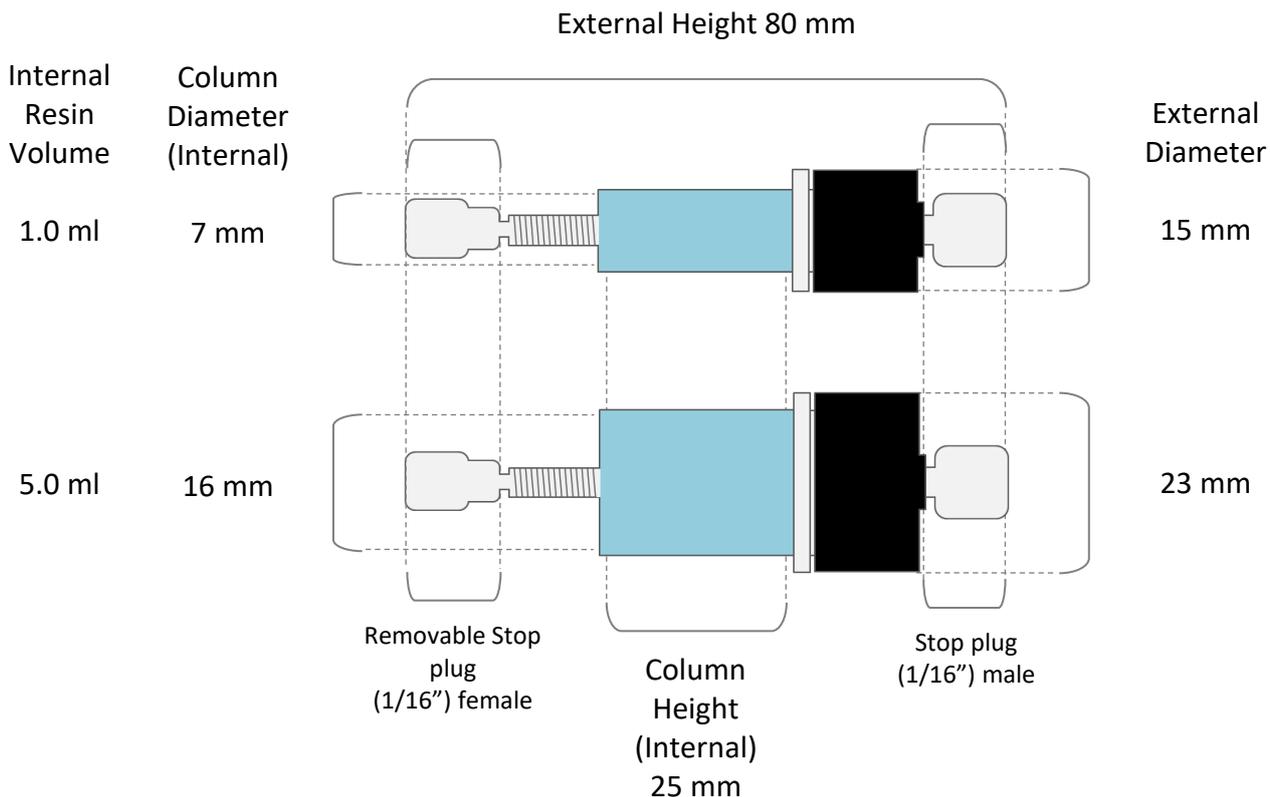


#### Features of the HiFliQ AAVX Short Bed FPLC columns:

- Fast and reliable affinity purification.
- Pre-packed 1 ml and 5 ml columns with pre-charged Nickel-NTA Agarose resin.
- 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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**HiFliQ 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.

**Resin Specification**

Column Characteristic	Description
Resin Support Matrix	Cross-linked poly(styrene-divinylbenzene)
Resin Ligand	Single domain (VHH) antibody fragment
Binding Capacity	>1x10 <sup>13</sup> 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	1ml/min (1ml) 5ml/min (5ml)
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<sub>2</sub>)
- <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 lifetime, 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
HiFliQ1-AAVX-1-SB	1ml AAVX HiFliQ column, short bed height 7mm ID x 25mm BH (1 x 1ml)	1
HiFliQ1-AAVX-5-SB	1ml AAVX HiFliQ column, short bed height 7mm ID x 25mm BH (5 x 1ml)	5
HiFliQ5-AAVX-1-SB	5ml AAVX HiFliQ column, short bed height 16mm ID x 25mm BH (1 x 5ml)	1
HiFliQ5-AAVX-5-SB	5ml AAVX HiFliQ column, short bed height 16mm ID x 25mm BH (5 x 5ml)	5

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