AAV Affinity Beads 4FF

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1. Product Description

AAV Affinity Beads 4FF is an affinity chromatography medium for the purification of adeno-associated virus (AAV) with high affinity for several subtypes of AAV. The base matrix of **AAV Affinity Beads 4FF** is highly cross-linked agarose derivative with excellent chemical and physical stabilities, making it ideal for process scale applications.The characteristics are summarized in table 1.

Table 1. Characteristics of **AAV Affinity Beads 4FF**

Item	Description
Matrix Spherical	Highly cross-linked 4% agarose
Ligand density	7 mg/ml
Binding Capacity	＞$1×10^{13}$ genome copies/mL medium
Particle size	45-165um
Maximum Pressure	0.3MPa, 3 bar
Storage Solution	1×PBS containing 20% ethanol
Storage Temperature	2 - 8℃

2. Virus Preparation

The production of AAV virus begins with the construction, expression and purification of the plasmid, as well as the recovery and expansion of mammalian or insect cells. After all the raw materials are ready, the transfection can be carried out. During the continuous culture process of transfected cells, the production of viral vectors also gradually begins in the cell. The cell culture method is different for different scale production, where adherent cells are suitable for the process development stage or are relatively easy to scale up, and the production method can use plates or cell factories. The large-scale production of most of the cells that choose suspension growth, because the suspension cell culture density is larger, easier to expand the culture volume, can use animal cell fermenter or wave reactor, with disposable culture consumables, can be stable, safe, fast and high-quality culture. The recombinant virus products obtained by the above culture methods can be chromatographed with **AAV Affinity Beads 4FF** in different scales.

3. Purification Procedure

3.1 Buffer Preparation

It is recommended to filter the water and buffers by passing them through a 0.22μm or 0.45 μm filter before use.
Binding/Wash buffer: 20 mM Tris-HCl, 0.5 M NaCl, pH 8.0
Elution buffer: 0.1 M citric acid-sodium citrate, 0.5 M NaCl, pH 2.5
Neutralization Buffer: 1 M Tris-HCl, pH 9.0
Note: In order to improve the elution purity, especially when there is interaction between the impurity protein and the target protein, the secondary washing operation can be added. The washing buffer can be adjusted as follows:

- Increase the concentration of sodium chloride to 1 M.
- Select a different pH wash buffer.
- Add Tween-20 with a final concentration of 0.05%.
- Add of ≤0.2 M $MgCl_2$ (avoid buffers containing phosphate to prevent precipitation from clogging the column).
- Add of ≤ 20% ethanol, which dose not damage the capsid structure of most serotypes.

3.2 Sample Preparation

The collected cell culture supernatant or cell lysis product needs to be centrifuged to remove cell debris or lysate impurities before loading the sample. It is recommended to add Benzonase Nuclease to degrade host nucleic acid. Finally, 0.22μm or 0.45μm filter membrane is

used before loading the sample to reduce impurities, improve protein purification efficiency and prevent blocking the column.

Adding Benzonase Nuclease can reduce nucleic acid impurities, reduce the viscosity of sample solution, reduce the pressure on chromatographic column during purification, reduce the host nucleic acid residue of purified sample, and improve the final sample purity.

3.3 Packing Columns

AAV Affinity Beads 4FF is easy to pack and use, and its high flow properties make it excellent for industrial scaling-up. The method of packing the column is described below.

1) Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

2) Close the column outlet leaving the net covered with packing buffer.

3) Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4) Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **AAV Affinity Beads 4FF** is packed at a constant pressure of approximately 3 bar (0.3 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5) Maintain packing flow velocity for at least 3 bed volumes. When the bed has stabilized, mark the bed height on the column and close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6) With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark, allowing the packing solution to flush the adapter inlet. Lock the adapter in position.

7) Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

8) Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

3.4 Sample purification

1) Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.

2) Wash the column with 10 column volumes of binding buffer.

3) Load the sample by using a syringe fitted to the connector or by pumping it onto the column.

4) Wash the column with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

5) Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

The elution need to be neutralized immediately. It is recommended to use Neutralization Buffer with 1/10 volume of the elution for neutralization.

3.5 SDS-PAGE assay

Identify the fractions using SDS-PAGE.

4. Cleaning-in-Place

AAV Affinity Beads 4FF should be cleaning-in-place after each purification. The recommended cleaning method is to use low pH buffer first and then denaturing agent.

The procedure is as follows:

- 5 column volumes 0.1 M phosphoric acid, pH 2.0 contact for 10 min.
- 5 column volumes 1× PBS, ph 7.4.
- 5 column volumes 6 M guanidine hydrochloride contact cleaning for 15 min.
- 15 column volumes 1 x PBS, pH 7.4.
- 3column volumes 1XPBS containing 20% ethanol, stored at 2-8°C.

5. Related Products

Product	Cat. No.	Size
AAV Affinity Beads 4FF	SA096005	5 ml
	SA096025	25 ml
	SA096100	100 ml
	SA09601L	1 L