Capto AVB

AFFINITY CHROMATOGRAPHY

Capto AVB™ is an affinity chromatography resin designed for the purification of adeno-associated virus (AAV). Compared with its predecessor AVB Sepharose™ High Performance resin, Capto AVB is based on a more rigid base matrix, delivering excellent pressure-flow properties to AAV production. The possibility to run at higher flow rates and bed heights increases flexibility in process design and allows for an increased productivity. Capto AVB is available in bulk as well as in formats suitable for process development (Fig 1).

Key benefits of Capto AVB include:

- Efficient, industrial-scale purification of AAV of several subclasses by affinity chromatography
- Non-mammalian derived product to reduce regulatory concerns
- High selectivity and excellent scalability

Fig 1. Capto AVB affinity chromatography resin designed for industrial purification of AAV.

Product overview

AAV is of increasing interest as a potential vector for gene therapy. To enable the use of AAV in clinical applications, an efficient and high-quality production process is needed, including downstream purification. The purification process should be robust, with high yields, high purity, and low ligand leaching. In current purification protocols, density gradient centrifugation is typically used, followed by several chromatography steps, giving a process with low yield and poor scalability.

When using Capto AVB, the AAV can be applied directly from clarified AAV vector cell lysate. Conventional buffers (e.g., PBS, Tris, citrate) can be used for loading, washing, and elution. Virus binds to the column at around neutral pH and is typically eluted by lowering the pH, for example, in the range of pH 2 to 5. As AAV is sensitive to highly acidic conditions (1), it is important to minimize the exposure to low pH during elution. Therefore, collected elution fractions should be neutralized immediately.

Principles of Capto AVB affinity chromatography

Affinity chromatography is one of the chromatographic methods for purification of a specific molecule or a group of molecules from complex mixtures. The technique offers high selectivity and usually high capacity for the target molecule. As affinity chromatography is a binding technique, the sample volume does not affect the separation. Diluted samples can be applied, although capacity is commonly somewhat lower with more diluted sample.

The immobilized ligand adsorbs the target molecule under suitable binding conditions. Under suitable elution conditions, the target molecule is desorbed. These conditions depend on the target molecule and feed composition, and should be evaluated together with other chromatographic parameters (e.g., sample load, flow velocity, bed height, regeneration, cleaning-in-place, etc.) to establish the conditions that will bind the largest amount of target molecule in the shortest time and with the highest product recovery.

While AAV is efficiently eluted at low pH, the virus is sensitive to highly acidic conditions. Although a somewhat lower recovery, studies have shown that a high pH elution buffer containing 0.5 to 1.0 M arginine also yields highly pure AAV, offering a viable alternative for purifying virus that is sensitive to low pH.

Bead size optimized for high-flow processes

Capto AVB is based on the well-established Capto high-flow agarose base matrix, which demonstrates excellent pressure-flow properties. The rigid matrix allows for high flow velocities in modern downstream purification processes (Fig 2). The ligand is attached to the base matrix via a hydrophilic spacer arm to make it easily available for binding of the virus (Fig 3).

The AAV affinity ligand was developed with technology from BAC BV (now part of Thermo Fisher Scientific Inc.). Ligand manufacturing, including fermentation and subsequent purification/formulation, is performed in the absence of mammalian components. The ligand itself was developed using single-domain antibody fragments directed against the target AAV. The gene of the selected protein was cloned into a yeast cell expression system.

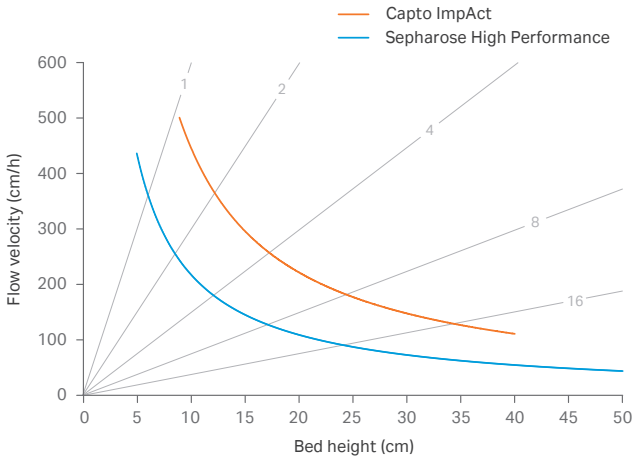

Fig 2. The window of operation (area under the curve) of Capto ImpAct (Capto AVB) and Sepharose High Performance (AVB Sepharose High Performance). Data correspond to a 1 m diameter column at 20°C and viscosity equivalent to water. Gray contours show the residence time in the column in minutes.

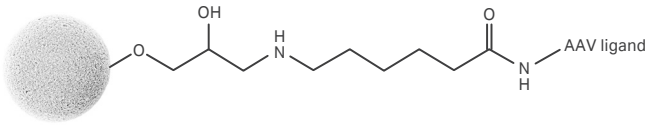

Fig 3. Partial structure of Capto AVB.

Process scale-up

Capto AVB is a BioProcess™ resin specifically designed to meet the demands of industrial applications. The resin is available prepacked in HiTrap™ columns suitable for process development and optimization as well as for laboratory-scale preparative purifications. Capto AVB is also available in bulk for scale-up to manufacturing scale.

Capto AVB is produced with reliable manufacturing procedures, and can withstand standard cleaning-in-place (CIP) and sanitization-in-place procedures. As a BioProcess resin, Capto AVB is supported with regulatory support files and comprehensive documentation, as well as security of supply services.

Capto AVB provides reproducibility and scalability, with purity levels that meet gene therapy standards. For scale-up of the AAV purification process, the recommended strategy is to optimize conditions using the prepacked Capto AVB columns, and then transfer the process to larger-scale columns (Fig 4). Even though the change in bed dimensions might be significant, only minor modifications to the conditions will be needed to optimize the purification.

Fig 4. Capto AVB processes for purification of AAV can be scaled from laboratory to production scale.

Productivity

A more rigid agarose resin allows for increased flow rates as well as the possibility to pack higher column beds, both enabling improved productivity. Increasing flow rate over the whole chromatographic purification process, (i.e., during column packing, conditioning, loading, washing, elution, regeneration, cleaning-in-place, and reconditioning) can substantially reduce total processing time. Using higher column beds with the same diameter, more viral vectors can be purified during the same cycle, which increases throughput. For example, going from a 15 cm bed height (AVB Sepharose High Performance) to a 20 cm bed height (Capto AVB) results in a 33% increase in resin volume and consequently 33% more product can be processed per cycle if the capacity of the resins is the same. Figures 5 and 6 shows the performance of Capto AVB in comparison with AVB Sepharose High Performance at 153 cm/h. At the higher flow velocity of 250 cm/h, the higher back pressure prevented the use of AVB Sepharose High Performance (Fig 7). Altogether, the use of a more rigid chromatography resin, such as Capto AVB, results in a significant improvement in downstream process productivity.

Column:	Capto AVB packed in Tricorn 5/20 (~ 400 µL)
Sample:	AAV crude cell lysate and supernatant
Sample load:	100 mL
Equilibration, loading, and wash buffer:	20 mM Tris-HCl, pH 7.5 + 0.5 M NaCl
First elution buffer:	100 mM sodium acetate, pH 2.5 + 0.5 M NaCl
Second elution buffer:	100 mM phosphate, pH 1.7 + 0.5 M NaCl
Flow velocity:	153 cm/h
System:	ÄKTA™ pure 25 M1

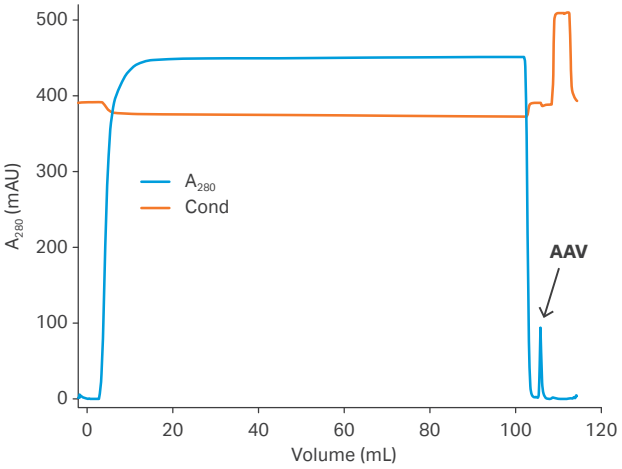

Fig 5. Purification of AAV on Capto AVB at a flow velocity of 153 cm/h. Chromatogram is based on data from 4D Molecular Therapeutics.

Column:	AVB Sepharose High Performance packed in Tricorn 5/20 (~ 400 µL)
Sample:	AAV crude cell lysate and supernatant
Sample load:	100 mL
Equilibration, loading, and wash buffer:	20 mM Tris-HCl, pH 7.5 + 0.5 M NaCl
First elution buffer:	100 mM sodium acetate, pH 2.5 + 0.5 M NaCl
Second elution buffer:	100 mM phosphate, pH 1.7 + 0.5 M NaCl
Flow velocity:	153 cm/h
System:	ÄKTA pure 25 M1

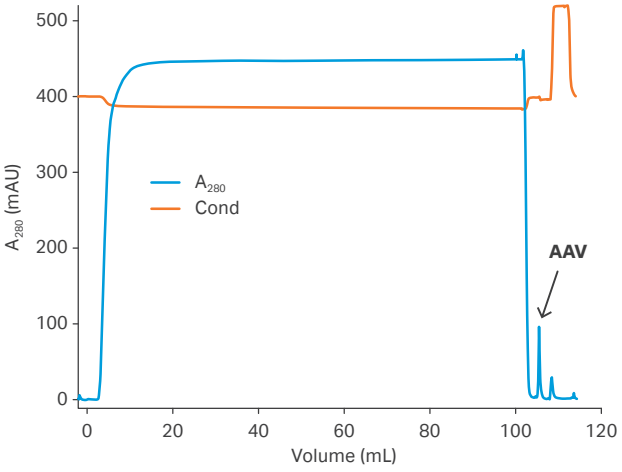

Fig 6. Purification of AAV on AVB Sepharose High Performance at a flow velocity of 153 cm/h. Chromatogram is based on data from 4D Molecular Therapeutics.

Column:	Capto AVB packed in Tricorn 5/20 (~ 400 µL)
Sample:	AAV crude cell lysate and supernatant (~ 10^9 viral genomes/mL)
Sample load:	650 mL
Equilibration, loading, and wash buffer:	20 mM Tris-HCl, pH 7.5 + 0.5 M NaCl
First elution buffer:	100 mM sodium acetate, pH 2.5 + 0.5 M NaCl
Second elution buffer:	100 mM phosphate, pH 1.7 + 0.5 M NaCl
Flow velocity:	250 cm/h
System:	ÄKTA pure 25 M1

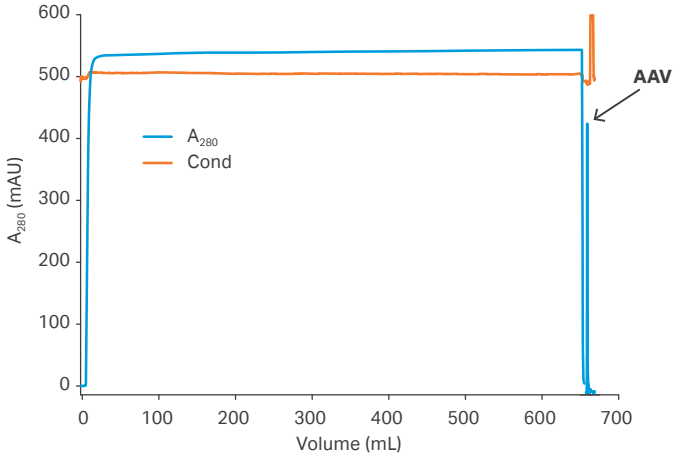

Fig 7. Purification of AAV on Capto AVB at a flow velocity of 250 cm/h. Chromatogram is based on data from 4D Molecular Therapeutics.

Product specifications

The main characteristics of the resin and columns are summarized in Tables 1 and 2, respectively.

Table 1. Characteristics for Capto AVB resins

Matrix	Highly cross-linked agarose, spherical
Ligand	Recombinant protein (Mr 14 000) produced in Saccharomyces cerevisiae. Binds AAV of subclasses 1, 2, 3, and 5
Ligand concentration	1.2 to 3.7 mg/mL
Particle size, d50V*	50 µm
Dynamic binding capacity†	Typically, > 1012 genome copies/mL of chromatography resin
Recommended flow velocity	Min. 220 cm/h‡
pH stability, operational§	3 to 10
pH stability, CIP¶	2 to 12
Chemical stability	Stable in commonly used aqueous buffers used in purification of AAV.
Delivery conditions	20% ethanol
Storage	20% ethanol at temperatures between 4°C and 8°C.

* Median particle size of the cumulative volume distribution.

† Conditions for determining dynamic binding capacity (DBC):
- Sample: AAV crude cell lysate and supernatant, approximately 10^9 viral genomes/mL
- Column volume: 400 µL (Tricorn™ 5/20)
- Flow rate: 250 cm/h
- Equilibration, loading and wash buffer: 20 mM Tris-HCl, 0.5 M NaCl, pH 7.5
- Elution buffer 1: 100 mM NaOAc, 0.5 M NaCl, pH 2.5
- Elution buffer 2: 100 mM H3PO4, 0.5 M NaCl, pH 1.7

‡ Determined at < 3 bar (44 psi, 0.3 MPa) in a 1 mL column at 20 cm bed height, measured at 20°C using process buffers with the same viscosity as water.

§ pH range where resin can be operated without significant change in function.

¶ pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function.

Table 2. Column characteristics

	HiTrap, 1 mL	HiTrap, 5 mL
Column volume	1 mL	5 mL
Column dimensions	0.7 × 2.5 cm	1.6 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)	5 bar (0.5 MPa)
Recommended operating flow rate*	1 mL/min	5 mL/min
Maximum operating flow rate*	< 4 mL/min	< 20 mL/min

* At room temperature in buffers with the same viscosity as water at 20°C.

Leakage assay

For determination of ligand leakage from Capto AVB, the Thermo Scientific™ CaptureSelect™ AVB Sepharose HP Leakage ELISA Kit (Thermo Fisher Scientific) can be used.

Cleaning and sanitization

A cleaning or sanitization protocol should be designed for each application, as the efficiency of the protocol is strongly related to the feedstock and other related operating conditions.

The recommended protocol comprises initial strip of the resin at low pH, and then subjecting the resin to NaOH of low concentration for cleaning. Lastly, PAB (120 mM phosphoric acid, 167 mM acetic acid, 2.2 % v/v benzyl alcohol) is used for final sanitization of the resin. PAB solution is sensitive to light and should be freshly made not to damage the resin.

PAB solution should be stored in a dark bottle and kept no longer than for a week. PAB solution has a pH of < 2, and resin stability can be limited in prolonged exposure at such a low pH.

1. 0.1 M citric acid, pH 2.1; 10 min; 13 CV 10 CV PBS, pH 7.4
2. 10 mM NaOH, pH 12; 15 min; 19 CV 10 CV PBS, pH 7.4
3. PAB; 15 min; 19 CV

Equilibrate the resin using equilibration buffer prior to next purification cycle.

Acknowledgment

We thank 4D Molecular Therapeutics for kindly sharing data and for valuable discussions.

Reference

1. Wu, N. *et al*. Production of viral vectors for gene therapy applications. *Curr*. Opin. *Biotechnol*. **11**, 205–208 (2000).

Ordering information

Product	Size	Product code
Capto AVB	25 mL	17372201
	100 mL	17372202
	1 L	17372203
	5 L	17372204
HiTrap Capto AVB	5 × 1 mL	17372211
	1 × 5 mL	17372212

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