AVB Sepharose High Performance

AVB Sepharose™ High Performance is an affinity medium designed for the purification of adeno associated virus (AAV). Key characteristics of AVB Sepharose High Performance include:

- Fast, one-step purification of adeno associated viruses of several subclasses
- Prepacked HiTrap™ columns for simple operation with a syringe, pump, or chromatographic system
- Excellent scalability

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

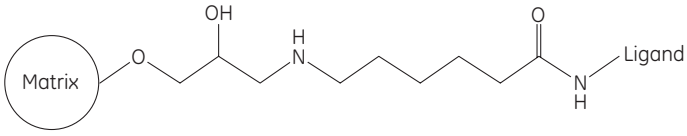

Fig 1. Partial structure of AVB Sepharose High Performance.

Table 1. Main characteristics of AVB Sepharose High Performance

Matrix	Cross-linked 6% agarose
Average particle size	34 µm
Ligand	14 kD recombinant protein produced in *S. cerevisiae*. Binds AAV of subclasses 1, 2, 3, and 5
Capacity	Typically > 10^{12} genome copies/mL gel
Recommended flow rate	Up to 150 cm/h at 30-cm bed height
Maximum back pressure	0.3 MPa, 3 bar
pH stability	
Long term	3–10
Short term	2–12

Medium characteristics

AVB Sepharose High Performance is based on a highly cross-linked 6% agarose matrix, which enables rapid processing of large sample volumes. The ligand, a 14 kD recombinant protein, is attached to the base matrix via a long, hydrophilic spacer arm to make it easily available for binding of the virus (Fig 1).

Table 1 summarizes the main characteristics of AVB Sepharose High Performance.

Functional Principles

Affinity chromatography exploits an immobilized ligand that adsorbs a specific molecule or group of molecules under suitable binding conditions and desorbs them under suitable elution conditions. These conditions depend on the target molecule, feed composition, and chromatography medium, and must be studied together with other chromatographic parameters (e.g., sample load, flow velocity, bed height, regeneration, cleaning-in-place) to establish the conditions that will bind the largest amount of target molecule, in the shortest time and with the highest product recovery.

When using AVB Sepharose High Performance the AAV can be applied directly from clarified AAV vector cell lysate. Typically loading, washing, and elution of the virus of the column is applicable with conventional buffers (e.g., PBS, tris, citrate). Binding of the virus to the column occurs around neutral pH and elution of the virus is done by lowering pH, for example to pH 2 to 5. Since AAV is sensitive to very acidic conditions, it is important to minimize the exposure to low pH during elution. Therefore it is recommended that collected elution fractions are neutralized immediately.

Regeneration should restore the original function of the medium. Depending on the nature of the sample, regeneration is normally performed after each cycle, followed by re-equilibration in start buffer. To prevent build up of contaminants over time, more rigorous protocols may have to be applied (see Cleaning-in-place and sanitization).

Stability

The ligand is linked to the Sepharose High Performance base matrix via a stable amide bond. In a study where AVB Sepharose High Performance was stored at room temperature at different pH values for one week it was shown that the leakage is low between pH 2 and 12 (Fig 2). At higher pH there is a leakage of both carbon and nitrogen, indicating hydrolysis of the ligand.

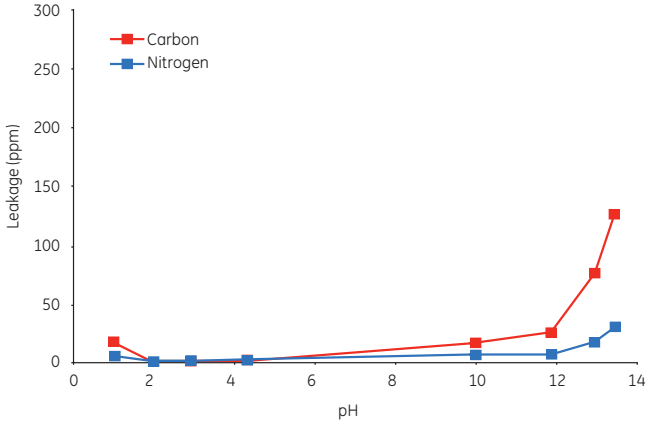

Fig 2. Stability of AVB Sepharose High Performance at different pH.

Cleaning-in-place and sanitization

A cleaning or sanitization protocol has to be designed for each application. A suggested cleaning protocol is to use a solution of low pH, for example 0.1 M phosphoric acid, alone or in combination with sodium chloride or ethanol. However, prolonged exposure (i.e., several days) to pH < 2 should be avoided due to a slow decomposition of the agarose matrix at low pH. Sodium hydroxide should be used with care due to the low stability under alkaline conditions.

Storage

The recommended storage conditions are 20% ethanol at 4°C to 8°C. AVB Sepharose High Performance is supplied pre-swollen in a 20% ethanol solution.

Ordering information

Product	Quantity	Code no.
AVB Sepharose High Performance*	75 mL	28-4112-01
AVB Sepharose High Performance*	1 L	28-4112-02
Prepacked HiTrap column*	5 x 1 mL	28-4112-11
Prepacked HiTrap column*	1 x 5 mL	28-4112-12

* This product is part of our Custom Designed Media program. If you are interested in large-scale quantities, please contact your local GE Healthcare representative.

Literature

Affinity Chromatography Handbook	18-1022-29
Affinity Columns and Media, Selection Guide	18-1121-86

www.gelifesciences.com/protein-purification-bioprocess

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Ltd
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327, Piscataway,
NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku,
Tokyo 169-0073, Japan

Asia Pacific T +85 65 62751830 F +85 65 62751829 • Australasia T +61 2 8820 8299 F +61 2 8820 8200 • Austria T 01 /57606 1613 F 01 /57606 1614 • Belgium T 0800 73 890 F 02 416 8206 • Canada T 1 800 463 5800 F 1 800 567 1008 • Central & East Europe T +43 1 972 720 F +43 1 972 722 750 • Denmark T +45 70 25 24 50 F +45 45 16 2424 • Eire T 1 800 709992 F +44 1494 542010 • Finland & Baltics T +358 9 512 3940 F +358 9 512 39439 • France T 01 69 35 67 00 F 01 69 41 98 77 Germany T 0800 9080 711 F 0800 9080 712 • Greater China T +852 2100 6300 F +852 2100 6338 • Italy T 02 26001 320 F 02 26001 399 • Japan T 81 3 5331 9336 F 81 3 5331 9370 • Korea T 82 2 6201 3700 F 82 2 6201 3803 • Latin America T +55 11 3933 7300 F +55 11 3933 7304 • Middle East & Africa T +30 210 96 00 687 F +30 210 96 00 693 • Netherlands T 0800-82 82 82 1 F 0800-82 82 82 4 • Norway T +47 815 65 777 F +47 815 65 666 • Portugal T 21 417 7035 F 21 417 3184 • Russia, CIS & NIS T +7 495 956 5177 F +7 495 956 5176 • Spain T 902 11 72 65 F 935 94 49 65 • Sweden T 018 612 1900 F 018 612 1910 • Switzerland T 0848 8028 10 F 0848 8028 11 • UK T 0800 515 313 F 0800 616 927 • USA T +1 800 526 3593 F +1 877 295 8102

imagination at work

28-9207-54 AA 03/2007