Data sheet

SepFast™ Mixed-Mode AH-1 Columns

SepFast MM AH-1 is a mixed mode chromatography medium that is supplied in a ready-to-use disposable column format. The key benefits are high binding capacity, high selectivity and low cost.

1. Properties

The ligand contains a combination of anionic and hydrophobic groups. The product shows good binding capacity to molecules rich in hydrophobic moieties. It shows little binding to DNA or albumins under moderate ionic strength (e.g. 0.15 M salt).

The base matrix is made of porous agarose. The particles have an open pore structure with good mass transfer properties to large protein molecules. The particle size range is 30 – 75 μm. It shows fast accessibility to both small and large protein molecules.

The medium shows high mechanical rigidity, so it can be operated at high flow velocities with moderate pressure drops. The medium is compatible with most of the chemical reagents commonly used in biological systems. It can be cleaned with 1M sodium hydroxide.

Table 1: Characteristics of SepFast MM AH-1 Columns:

Matrix	Agarose
Functional group	Mixed-mode anionic and hydrophobic groups
Small ionic capacity	0.08 – 0.2 mmol Cl^-/ml medium
Column material	Polypropylene (end-caps, meshes, stop plungers), acrylic or polypropylene (column body), NBR O-rings
Operational pressure	Up to 3 bar
Column pressure	4 bar
pH stability	2-14 (short term) and 4-12 (long term)
Working temperature	+4^{O}C to +30^{O}C
Chemical stability	Compatible with commonly used buffers
Avoid	Alcohols (>20%, if acrylic column body used), oxidizing agents, anionic detergents

2. Applications

SepFast MM AH-1 can be used for the selective purification of antibody fragments and other recombinant proteins under moderate ionic strength. The medium could be used in the final polishing step to remove impurities e.g. viruses, host cell proteins and endotoxins. The feedstock containing the target protein can pass through the column at high velocity with much improved binding capacity.

SepFast MM AH-1 could also be explored for the cost-effective separation of antibody monomers from their aggregates.

3. Operations

The packed resin is stored in 20% ethanol or in 0.02% sodium azide solution on delivery. It can be directly connected to a suitable chromatography system such as AKTA. Normally, the end with the product label should be connected as the top inlet. If there is no label difference between those two ends, the column can be connected either way.

The column need be equilibrated with at least 5 – 10 column volumes of the equilibration buffer until the pH and conductivity signals become stable, before a sample is loaded.

The running pressure shall not exceed 3 bar during the operation.

After each application, seal the column ends and store it properly if re-use is expected.

4. Method optimization

We recommend scouting for optimal binding / elution pH and for optimal ionic strength. Typically, SepFast MM AH-1 binds proteins at neutral pH and moderate salt conditions (e.g. 0.15 M NaCl). The elution is achieved by reduced pH or salt concentration. Due to the fast pore accessibility, the binding step could be done at a faster flow velocity. We recommend paying special attention to optimising elution conditions to achieve improved purity in the elution step.

In general, balancing product recovery against process throughput is the major consideration when optimizing a method. However, for the purification of shearing-force sensitive molecules, the operational flow velocity needs to be optimised to balance the throughput and minimise the possible damage to the target molecule.

5. Process scaling up

SepFast MM AH-1 pre-packed columns range from small to large to support the scaling up work. Please contact us for further information.

6. Maintenance

Depending on the individual applications, the column may be single use or re-used. For the re-use purpose, please see the following instructions.

Regeneration

After each run, elute any reversibly bound material either with sodium hydroxide or reduced pH.

Cleaning-in-place (CIP)

CIP is a procedure that removes strongly bound materials such as lipids, endotoxins and denatured proteins that remain in the column after regeneration. Regular CIP prevents the build up of contaminants in the packed bed and helps to maintain the column performance.

A specific CIP protocol should be developed for each process according to the type of contaminants present. The frequency of CIP depends on the nature of individual applications.

The following information works as a general guide.

Salt of concentrations up to 2 M can be used to clean the impurities bound by ionic interactions. The contaminants bound hydrophobically can be removed by using the following reagents: 1 M NaOH, low percentage non-ionic detergents (e.g. 0.1 – 2%), 30% isopropanol in basic or acidic conditions (e.g. in the presence of acetic acid or phosphoric acid). A combination of the above reagents can be explored as well. In general, the incubation time should be longer (e.g. from 30 minutes to 2 hours) to ensure full dissociation of the contaminants. Long contact time should be avoided when alcohols are used, as the acrylic column body may be damaged.

Sanitization
Sanitization using 0.5-1.0 M NaOH with a contact time of 1 hour is recommended.

7. Storage

The column should be equilibrated in binding buffer containing 20% ethanol or 0.02% sodium azide to prevent microbial growth. Store the column at a temperature of +4°C to +30°C. After storage, equilibrate the column with at least 5 bed volumes of running buffer before use.

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