JSR Life Sciences

Description

Product name	Amsphere™ A3
Matrix	Methacrylic polymer
Average particle size	50 µm
Ligand	Recombinant protein A expressed in *Escherichia coli*
Dynamic binding capacity*1	Approximately 54 mg/mL for polyclonal IgG
Maximum operating pressure	0.8 MPa*2
Maximum operating velocity	1200 cm/h (dependent on column size)
Recommended bed height	5 - 25 cm
Working pH range	1 - 13
Cleaning-in-Place stability	0.1 - 0.5 M NaOH
Recommended storage buffer	50 mM sodium phosphate buffer containing 16% Ethanol, pH 7.5

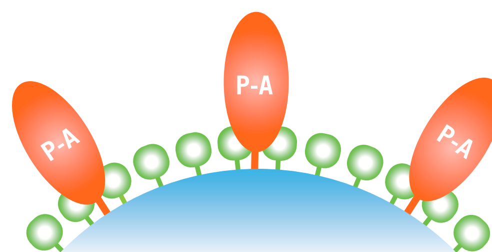
*1 Determined at 10% breakthrough under linear velocity of 300 cm/h in a column with bed height of 20 cm.
*2 Do not exceed the column's pressure resistance.

Operational Conditions

General
Ensure all buffers and the sample feedstock are 0.22 µm filtered before use.

For equilibrating the column, neutral buffer or buffers having a similar composition as the feedstock are preferred. For instance, 20 mM sodium phosphate, pH 7.5 or 25 mM Tris-HCl, pH 7.0 with 0-150 mM NaCl can be used.

Amsphere™ A3



Amsphere A3 is a new protein A resin designed with a surface modified base bead and alkali-resistant optimized ligand.

Protein A ligand

- High DBC via controlled conformation and orientation
- High alkaline stability from protein engineering

Surface modification

- Low HCP levels by surface hydrophilization

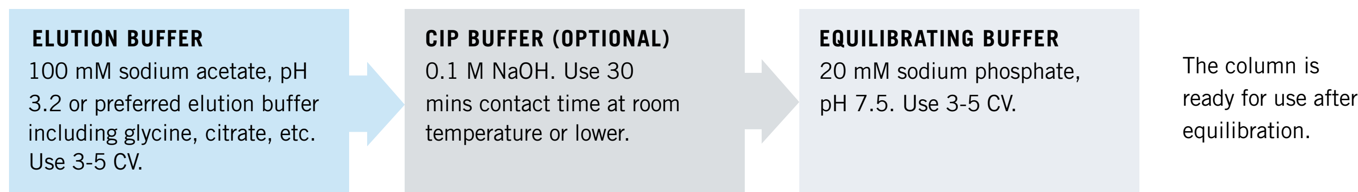
Base bead formulation

- High DBC at high flow rate
- Excellent pressure and flow properties via rigid crosslinking

Packing of Column and Evaluation

The packing protocol for Amsphere A3 is detailed in our “Column Packing Protocol for Amsphere A3” which is available from your JSR Life Sciences sales representative.

It is recommended to perform the following blank run before first use of the column.



Sample Loading

The capacity of the resin will depend on the antibody class, the salt concentration, the pH and flow rate of the load. It is therefore recommended to optimize the loading conditions on a small scale by measuring the chromatographic parameters, such as DBC, before scaling up the process.

Washing

The properties of the washing buffer such as volume, conductivity and pH, can be modified depending on the antibody load and end user’s preferred buffers. In cases where significant removal of host cell proteins (HCP) or deoxyribonucleic acids (DNA) is needed, use of the following buffers, in the suggested order, is recommended:

1. 20 mM sodium phosphate, pH 7.5, 1-2 CV
2. 20 mM sodium phosphate, 500 mM-1 M NaCl, pH 7.5, 4-6 CV
3. 20 mM sodium phosphate, pH 7.5, 2-4 CV

Sometimes the wash buffer can be at a lower pH buffer with high salt, followed by no salt buffer:

1. 100 mM sodium acetate, pH 6.0, 2 CV
2. 100 mM sodium acetate, 500 mM to 1M NaCl, pH 6.0, 4 CV
3. 100 mM sodium acetate, pH 6.0, 4 CV

Note: Salt wash (500 mM-1 M salt) is recommended for Amsphere A3

Elution

A general acidic buffer can be applied for elution. It is recommended to use a sodium acetate buffer, but sodium citrate or glycine may also be used. To determine the optimum pH for elution, a linear gradient may be performed at small scale. Collect fractions and neutralize with 1.0 M Tris and assay fractions for mAb product. Optimal pH conditions for peak mAb elution can be determined.

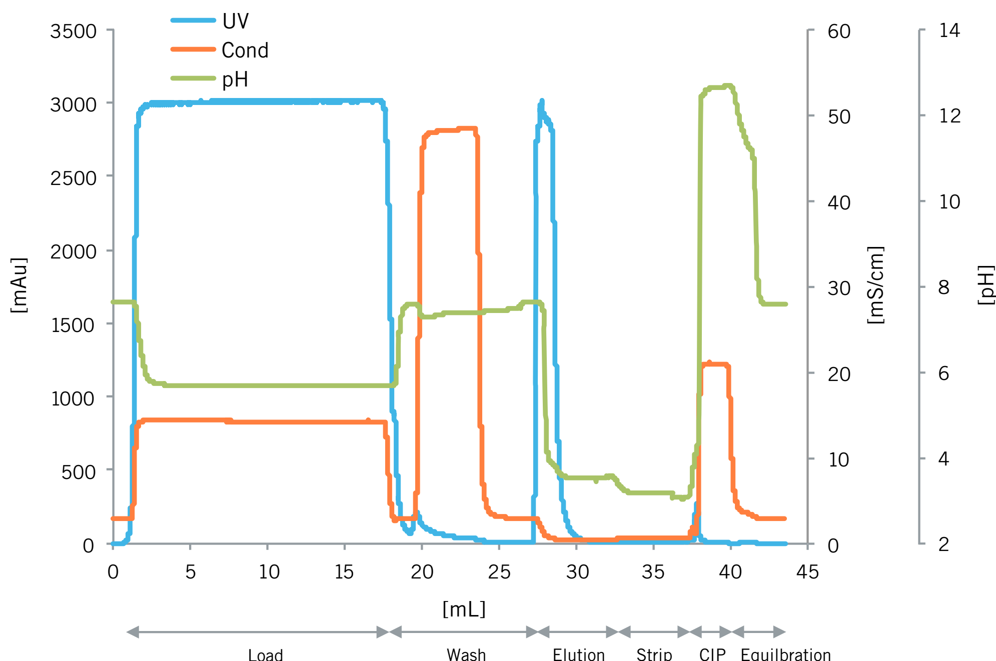
Regeneration

After antibody elution, 5 CV of a buffer that is more acidic than the elution buffer can be used to flush residue from the column. The use of an acetic, hydrochloric or phosphoric acid solution (pH 1.5-3.0, 20-200 mM) is preferred for this purpose.

Example of Chromatographic Run

Figure 1 shows an example of purification of mAb from a CHO harvested cell culture with Amsphere A3. A column with bed height of 5 cm and ID of 0.5 cm (1 mL) was used. The load was 44 mg mAb at 4 min residence time and the yield was 94% of highly purified antibody.

FIGURE 1: PURIFICATION OF mAb FROM A CHO HARVESTED CELL CULTURE WITH AMSPHERE A3



Cleaning-in-Place (CIP)

Typical conditions for CIP are as follows: 0.1 M to 0.5 M NaOH.

The recommended CIP protocol is as follows:

1. Wash the column with at least 2 CV of 0.1 M NaOH. A contact time of 15-30 minutes is sufficient and will depend on the antibody feedstock being purified.
2. Wash the column with approximately 5 CV of the equilibration buffer or until the pH stabilizes.

CAUTION:

- Do not apply a high flow rate as the pressure may increase when pumping NaOH solution over the column.
- Although Amsphere A3 is alkali-resistant, a long contact time with alkaline buffers can decrease DBC. Equilibrate the column with the equilibrating buffer immediately after CIP. Alkaline stability data is available from JSR Life Sciences.

Storage

The Amsphere A3 slurry is supplied in a sodium phosphate buffer containing 16% ethanol. The media should be stored between 2 - 8 °C and should not be frozen. For storing a column packed with Amsphere A3, equilibrate the column with a buffer containing 16 - 20% ethanol, and store between 2 - 8 °C. As an alternative, 2% benzyl alcohol can also be used. After long-term storage, it is recommended to do a blank run including CIP before use.

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