Agarose Bead Technologies

SIZE EXCLUSIOM CRHROMATOGRAPHY

Plain & Crosslinked Agarose Beads

DESCRIPTION

Plain and crosslinked agarose beads are used in Gel Filtration Chromatography (or Molecular Exclusion Chromatography) as well as for activating beads for biomolecule purification or immobilization.
Agarose beads can be used in column or batch format. Detailed below are some recommendations to consider in the column packaging and equilibration as well as in the subsequent sample application.

INSTRUCTIONS

The procedure for the correct packaging of the column is described below:

1. Manually shake the bottle to obtain a homogenous suspension of Plain or Crosslinked Agarose Beads and preservative. Place a funnel in the head of the column and slowly run suspension down the walls of the column.

Note: it is advisable to make the addition slowly to avoid formation of bubbles. The product may also be degassed before it is added to the column.

2. Repeat previous steps until the desired column height is obtained.

3. Insert the adapter gently in the column head until it begins to displace the liquid and make sure no air is trapped under the net.

4. Connect the pump to the column and watch that the column height remains the same as the flow of distilled water is passing through.

5. When a constant height has been obtained, maintain the flow with the addition of 5 volumes of distilled water to completely eliminate the preservative.

6. Equilibrate the column with 2 - 5 volumes of elution buffer.

Note: It is advisable to previously de-gas all the solutions before adding them to the column to avoid formation of bubbles. It is also advisable to add at least 0.2M of NaCl to the equilibration buffer to avoid ionic interactions.

7. It is advisable to utilize sample volumes of about 2-5% of the entire volume of the column.

8. For regeneration and later reuse of agarose beads, washing the column with 3 volumes before re-equilibrating with a new buffer is recommended.

Note: If poor resolution or strange pressures are observed, it is advised to insert a washing step before proceeding to the re-equilibration step. This washing step can be done at a high ionic strength (thus eliminating precipitated or nonspecifically bound proteins) or adding a non-ionic detergent.

It is advisable to keep the product in an appropriate preservative between uses.

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STABILITY PRODUCT	STUDIES	REAGENTS
Plain Agarose Beads	Thermical Stability	Not autoclavable
	Chemical Stability	Stable to all solutions commonly used in Gel Filtration. Including 8M urea and 6M guanidine hydrochloride. Oxidizing agents is not advisable. Stable in acid (pH 4.0) and basic (pH 9.0) solutions. Resistant to biological degradation.
	Physical Stability	Negligible volume variation due to changes in pH or ionic strength.
Crosslinked Agarose Beads	Thermical Stability	Autoclavable 30 minutes at 121ºC at pH 7.
	Chemical Stability	Stable to all solutions commonly used in Gel Filtration including 8M urea and 6M guanidine hydrochloride. Stable in organic solvents such ethanol, DMF, acetone, DMS, chloroform, dichloromethane, dichloroethane, pyridine, trethyl phosphate and acetonitrile. Oxidizing solutions should be avoided. Stable in strong acid (pH 2.0) and strong basic (pH 13.0) solutions. Dissociating agents and chaotropic salts (urea. Guanidine, KSCN, DMS or similar reagents) can be used. Resistant to biological degradation.
	Physical Stability	Negligible volume variation due to changes in pH or ionic strength.

GENERAL RECOMMENDATIONS

Depending on the objective pursued in each Gel Filtration, it is important to get a good resolution between peaks. If the objective is group separation (big proteins from small proteins), achieving a good resolution in all peaks it is not so important. However, if the objective is to fractionate different proteins from a mixture by their size, resolution between peaks is of vital importance.

Some recommendations to bear in mind for a good resolution are listed below:

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•Sample volume and column size are the most important factors that influence resolution.Generally speaking, volumes to apply when fractionating by size are less than 2% of the column volume. This quantity changes depending on the peaks resolution of interest). When separating by size (big molecules from small molecules), volumes 15 times bigger can be applied. In this technique dissolution effects are unavoidable, so it is recommended to work with the highest sample concentration that allows a good resolution.

A bigger sample volume requires a bigger column volume. In some cases, it is advisable to use smaller columns and repeat the experiment with different sample fractions.

•Other aspects that may influence separation are molecules to separate size distribution, molecule pore size, flow and column packaging.

•Sample viscosity. When the sample is viscous, it is advisable to consider the possibility of working at higher temperatures to reduce the viscosity.

•Buffer composition does not directly affect resolution. However, it is important to choose a buffer and a pH compatible with the protein stability/activity because it can affect biological form or activity of the molecules to separate. For example, pH, ionic strength or denaturing agents can produce conformational changes or protein dissociation.

It is recommended to use a buffer concentration enough to keep pH constant, and to add 0.15M of NaCl to avoid nonspecific interactions that would result in peak delays. A buffer that is commonly used is 0.05M phosphate, 0.15M NaCl and pH 7.0.

Note: It is advisable to previously de-gas the buffer and prepare it in high quality water.

•Denaturing agents and detergents: These agents can be used to solubilize the protein. However, they denature protein, so normally using them is avoided. If they are necessary, they should be present both in the running buffer and the sample buffer.

Note: A high detergent concentration results in a higher pressure making flow reduction necessary. In case of samples that contain these agents it would be necessary to include them also in calibration.

Tips for better chromatographic goals:

ANALYTICAL METHOD (top priority: maximum resolution):

•Beads as small as possible

•Column as large as possible

•Optimum gel pore size

•Small sample loading

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PREPARATIVE METHOD (top priority: maximum throughput):

- Beads as large as possible
- Column as short and wide as possible
- Optimum gel pore size
- Largest permissible sample loading
- Fastest flow rate (highest permissible pressure)

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