BCL Agarose Bead Standard (50-150 µm) (4%)

Catalogue No.:abx291353

4% BCL Agarose Bead Standard is a crosslinked agarose resin with a bead size of 50-150 µm. It is a gel filtration resin commonly used for coupling affinity ligands. The matrix is not pre-activated so the user needs to generate groups for coupling procedures.

Bead Geometry & Size	Spherical ~ 50-150 µm
Bead Mean Diameter d50v	~90 µm
Crosslinked	Yes
Agarose %	4%
Linear Recommended Flow Rate	< 26 cm/h
Fractionation (mw) Globular Proteins Da	7×10^4 - 2×10^7
Exclusion Limit Da	> 2×10^7
pH Stability Working Range	3-13
pH Stability Cleaning-In-Place (CIP)	2-14
Chemical Stability	Stable in all solutions commonly used in gel filtration, 2 M NaOH, 8 M urea, 6 M guanidine hydrochloride, 30% isopropanol, 70% Ethanol and commonly used detergents.
Antimicrobial Agent	20% Ethanol

Target: BCL Agarose Bead Standard (50-150 µm) (4%)

Storage: Store at 2-30°C.

Stability: Thermal stability: Autoclavable - 30 min @ 121 °C. pH 7.
Chemical stability: Stable in all solutions commonly used in gel filtration, including 8 M urea and 6 M guanidine hydrochloride. Stable in organic solvents such as 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.

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use:

Suggested Column Packing Procedure for Size Exclusion Chromatography

1. Shake the bottle to obtain a homogenous suspension. It is recommended to de-gas all solutions before adding them to the column to avoid formation of bubbles.
2. Place a funnel in the head of the column and slowly run the suspension down the walls of the column. Avoid bubbles. Repeat until the desired column height is obtained.
3. Insert the adapter gently in the column head until it begins to displace the liquid. Ensure that 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. The buffer should contain at least 0.2 M of NaCl to avoid ionic interactions.
7. Use sample volumes of about 2-5% of the entire volume of the column
8. For regeneration and later reuse of agarose beads, wash the column with 3 volumes before re-equilibrating with a new buffer. If poor resolution or strange pressures are observed, it is recommended 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 non-specifically bound proteins) or adding a non-ionic detergent. The product should be kept in an appropriate preservative between uses.

General Recommendations

• Sample volume and column size are the most important factors that influence resolution. Generally, volumes to apply when fractionating by size should be 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 larger 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. Other aspects that may influence separation are molecules to separate size distribution, molecule pore size, flow, and column packaging.

• If the sample is viscous, consider working at higher temperatures to reduce the viscosity.

• Buffer composition does not directly affect resolution. However, it is important to choose a buffer that is pH compatible with the protein stability/activity because it can affect biological form or activity of the molecules to be separated. For example, pH, ionic strength or denaturing agents can cause conformational changes or protein dissociation. It is recommended to use a buffer concentrated enough to keep pH constant, and to add 0.15 M of NaCl to avoid nonspecific interactions that would result in peak delays. A buffer that is commonly used is 0.05 M phosphate, 0.15 M NaCl (pH 7.0). Buffers should be degassed before use and prepared in high quality water.

• Denaturing agents and detergents can be used to solubilize the protein. However, as they denature proteins, it is recommended to avoid using them. If they are necessary, they should be present both in the running buffer and the sample buffer. Note that a high detergent concentration results in a higher pressure making flow reduction necessary. If samples contain these agents, it would be necessary to include them also in calibration.

• Recommendations for analysis (maximum resolution):
  - Beads as small as possible
  - Column as large as possible
  - Optimum gel pore size
  - Small sample loading

• Recommendations for 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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