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Agarose 6B

User's Guide

1. DESCRIPTION

Agarose 6B is a bead-formed agarose-based gel filtration matrix. It is also used for coupling affinity ligands to the matrix.

2. TECHNICAL SPECIFICATIONS

	Agarose 6B
Matrix	Agarose , 6%
Bead form	Spherical, diameter 50 µm-160 µm
pH stability Working Range	4-9
pH stability Cleaning-in-Place (CIP)	4-9
Maximum Pressure (MPa)	0.02
Maximum Flow Velocity	14 cm/h
Fractionation [Mr] Globular Proteins	1 x 10^4-4 x 10^6
Physical Stability	Negligible volume variation due to changes in pH or ionic strength
Chemical Stability	Stable to: 6 M urea, 8 M guanidine hydrochloride
Storage Conditions	4 to 30°C, 20% Ethanol

3. PREPARING THE MEDIUM

Agarose 6B is supplied in a solution containing 20% ethanol. These solution must be washed away before use.

4. PACKING AGAROSE 6B

Prepare a slurry with binding buffer in a ratio of 75% settled medium to 25% buffer.

4.1 Equilibrate all material to room temperature.

4.2 De-gas the slurry

4.3 Eliminate air from the column dead spaces by flushing the end pieces with buff. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buff remaining in the column.

4.4 Pour the gel slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.

4.5 Fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.

4.6 Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate is typically employed during packing.

4.7 Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adapter

4.8 After the medium have been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buff to form an upward meniscus at the top.

4.9 Insert the adaptor into the top of the column at an angle, taking care not to trap air under the net.

4.10 Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.

4.11 Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.

4.12 Lock the adapter in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adapter on the medium surface as necessary.

The column is now packed and equilibrated and ready for use.

5. OPERATION

5.1 Equilibration

Equilibrate the column with the starting buffer when the pH and/or conductivity of the effluent is the same as the starting buffer.

5.2 Sample preparation

Before application the sample should be centrifuged or filtered through a 0.45µm filter to remove any particulate matter. Recommended sample volumes is 2-5% of the total bed volume.

5.3 Elution

It is recommended to use a buffer with an ionic strength of 0.15 or greater to avoid any unwanted ionic interactions between the solute molecule and the agarose beads.

6. REGENERATION

After every run, elute reversibly bound material with low ionic strength buffer, and wash with H2O and starting buffer.

7. CLEANING-IN-PLACE(CIP)

Remove precipitated proteins and hydrophobically bound proteins or lipoproteins: Wash with 0.5 M NaOH and immediately rinse with eluent buffer.

Lipids and very hydrophobic proteins: Wash the column with non-ionic detergent , followed by at least 2-3 column volumes of eluent buffer.

8. SANITIZATION

Wash the column with 0.5M NaOH for 30–60 min. Sanitization is the use of chemical agents to inactivate microbial contaminants in the form of vegetative cells; it also helps to maintain a high level of both process hygiene and process economy.

9. STORAGE

Agarose 6B should be stored in the salt form in a buffer containing 20% ethanol. Recommended storage at 4 to 30°C. Do not freeze.

10. SHELF LIFE

5 year