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Dextrin XPure Agarose Resin

User's Guide

1. DESCRIPTION

Dextrin XPure Agarose Resin is a chromatography medium for the isolation of proteins fused to maltose binding protein (MBP-tagged protein). Tagging proteins with MBP often gives increased expression levels and higher solubility of the target protein. Proper folding of the attached protein has also been shown to be promoted by the MBP tag. Since MBP increase solubility, the tag is particularly useful for recombinant proteins accumulated in an insoluble form (inclusion bodies). See table 1. Affinity purification using **Dextrin XPure Agarose Resin** take place under physiological conditions and mild elution is performed using maltose which preserve target protein activity.

Table 1. Characteristics of **Dextrin XPure Agarose Resin**

Property	Description
Matrix	Highly cross-linked 6% agarose
Ligand	Dextrin
Capacity （/ml medium）	>10 mg MBP tagged protein(80 kDa)
Particle size (μm)	45-165
Maxi pressure	0.3 MPa, 3 bar
pH stability	3-12
Storage buffer	20% ethanol
Storage	2°C - 8°C

2. PURIFICATION PROCEDURE

2.1 Buffer Preparation

Water and chemicals used for buffer preparation should be high purity. It is recommended filtering the buffers by passing them through a 0.22μm or 0.45μm filter before use.

Binding /Wash Buffer: 20mM Tris-HCl, 200mM NaCl,1mM EDTA,pH7.4

Elution Buffer: 20mM Tris-HCl, 200mM NaCl,1mM EDTA, 10mM maltose ， pH7.4

Optional: 1mM DTT or 10mM β-mercaptoethanol

2.2 Sample Preparation

It is recommended filtering the sample solution by passing them through a 0.22μm or 0.45 μm filter

before use.

2.3 Packing Columns

1). Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net. 2). Close the column outlet leaving the net covered with packing buffer.

3). Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.

If using a packing reservoir, immediately fill the remainder of the column and reservoir with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4). Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, **Dextrin XPure Agarose Resin** is packed at a constant pressure of approximately 1 bar (0.1 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer).If the recommended pressure or flow velocity can not be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

5). When the bed has stabilized, close the bottom outlet and stop the pump.

If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

6). With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

7). Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

8). Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

2.4 Sample Purification

1). Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, “drop to drop” to avoid introducing air into the column. Remove the snap-off end at the column outlet.

2). Wash the column with 10 column volumes of binding buffer.

3). Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

4). Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

5). Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

2.5 Analysis

Identify the fractions containing the MBP-tagged protein. Use UV absorbance, SDS-PAGE, or western blot.

3. CLEANING-IN-PLACE

In general, **Dextrin XPure Agarose Resin** is well suited for reuse a number of times. When precipitation and protein aggregation cause the loss of velocity and combined loads, you need to clean the medium as follows.

3 column volumes deionized water;

3 column volumes 0.1% SDS or 0.5M NaOH solution；

3 column volumes deionized water；

Store at 2°C - 8°C with 20% ethanol.

4. TROUBLESHOOTING

Problem	Probable Cause	Solution
Back pressure is too high	Column is clogged	Cleaning in place(part 3)
	Sample solution contains precipitate	Filtering the sample solution by passing them through a 0.22μm or 0.45 μm filter.
No binding	Expression of target protein is very low	Check expression level of protein by estimating the amount in the extract, flow through, elute fraction and pellet upon centrifugation. Or apply large sample volume.
	There are some interference factors in the sample or buffer.	Sample dialysis or diluted with binding buffer.

	Amylase produced by cells affected the protein combined with the medium.	Inhibit the expression of amylase by adding glucose to the culture medium.
	Contact time is too short.	The sample and the medium was incubated for 2 hours at RT or longer.
The elute is not pure	Protein degradation	Add some protease inhibitors, such as PMSF, EDTA.
	Wash is not enough	Increase the volume of Wash Buffer.