Cyrusbioscience

Amylose Dextrin Resin

Cat. Number: ADR

Storage: Store at 4°C.

Description

Amylose Dextrin Resin is a chromatography medium for purifying proteins fused to maltose binding protein (MBP-tagged protein). Recombinant proteins with MBP-tags 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 the solubility, the tag is extremely useful for recombinant proteins accumulated in an insoluble form (inclusion bodies).
The base matrix of Amylose Dextrin Resin is a robust, highly cross- linked 6% agarose. The crosslinking of the base matrix has been optimized to give high physical and chemical stability, high specificity of the binding.

Affinity purification using Amylose Dextrin Resin take place under physiological conditions and mild elution is performed using maltose which preserve target protein activity. These mild conditions may even allow purification of intact protein complexes.

Characteristics of Amylose Dextrin Resin

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

Purification Procedure

Buffer Preparation:

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

Sample Preparation:

1. Harvest cells from appropriate volume of bacterial culture by centrifugation at 7,000rpm for 10-15min at 4°C, and discard the supernatant.
2. Resuspend cell pellet in 1:10 ratio(w/v) in Binding/Wash Buffer by pipetting or inverting at room temperature (20–25°C) or 4°C.
3. Disrupt resuspended cells using sonication in short bursts.
4. Centrifuge lysed cells at 12,000 × g for 40 minutes at 4°C. Transfer the supernatant to a new tube. Reserve a fraction of supernatant and pellet for SDS-PAGE analysis to determine solubility of the protein.

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, Amylose Dextrin 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.

Sample Purification:

1. Fill the syringe or pump tubing with equilibration 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 equilibration 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 wash 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.

Analysis:

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

Cleaning-in-Place

In general, Amylose Dextrin 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.

1. Wash with 3 column volumes deionized water;
2. Wash with 3 column volumes 0.1% SDS or 0.5M NaOH solution;
3. Wash with 3 column volumes deionized water;
4. Store at 2°C - 8°C with 20% ethanol. Re-equilibrate the column with 10 CV of binding buffer before starting the next purification.

Troubleshooting

Problem	Probable Cause	Solution
Back pressure is too high	Column is clogged	Cleaning in Place step
	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.