

CM XPure Agarose Resin

User's Guide

1. DESCRIPTION

CM XPure Agarose Resin is part of Ion exchange Resin which is widely used in biomedical and bioengineering for separation and purification of proteins, nucleic acids and polypeptides. The base matrix **CM XPure Agarose Resin** is 6% highly cross-linked agarose which gives the ion exchangers high chemical and physical stability. They are developed and supported for process scale chromatography. The characteristics such as capacity, elution behavior and pressure/flow rate are unaffected by the solutions commonly used in process chromatography and cleaning procedures, for details see table under each respective ion exchanger. Fig.1 is the pressure/flow rate curves of the matrix.

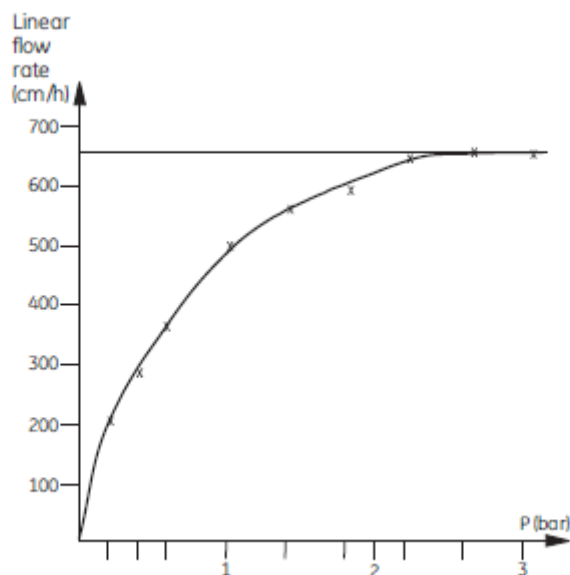


Fig.1. A typical pressure/flow rate curves for **CM XPure Agarose Resin**

CM XPure Agarose Resin is a weak cation exchanger. The ion exchange group is a carboxy methyl group, see below.

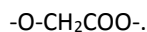


Table 1. Characteristics of **CM XPure Agarose Resin**

Property	Description
Matrix	Highly cross-linked 6% agarose



Ion exchange type	Weak cation
Total ionic capacity	0.09-0.13mmol H ⁺ /ml medium
Particle Size (µm)	45-165
Flow rate	300-600cm/h
pH stability	4-13
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.

2.2 Sample Preparation

It is recommended filtering the sample solution by passing them through a 0.22µm or 0.45µm 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, the medium 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 cannot 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 using UV absorbance, SDS-PAGE, or western blot.

3. CLEANING-IN-PLACE

After each separation, elute any reversibly bound material either with a high ionic strength solution (e.g. 1M NaCl in buffer) or by increasing pH. Regenerate the media by washing with at least 5 bed volumes of buffer, or until the column effluent shows stable conductivity and pH values.

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that may remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the media bed and helps to maintain the capacity, flow properties and general performance of the media.

A specific CIP protocol should be designed for each process according to the type of contaminants present. CIP cycle is generally recommended every 1-5 separation cycles.



Remove the ionically bound proteins

Wash with 1 column volumes of 2M NaCl. Contact time 10-15min.

Remove the precipitation or hydrophobically bound proteins or lipoproteins

Wash with at least 2 column volumes of 1M NaOH . Contact time 1-2h.

Remove lipids and very hydrophobic proteins

Wash with 3 column volumes of 0.5% non-ionic detergent (e.g.1M acetic acid),70% ethanol or 30% isopropanol. Contact time 1-2h.

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 0.45µm filter.
Elute is not pure	The medium repeat too much times.	Cleaning in place (part 3).
	Wash is not enough.	Increase the volume of Wash Buffer.

