GE Healthcare

Instructions 71-7076-00 AD **Affinity media**

Calmodulin Sepharose 4B

Calmodulin Sepharose™ 4B is calmodulin immobilized by the CNBr method to Sepharose 4B.

Calmodulin is a highly conserved regulatory protein found in all eukaryotic cells. This protein is involved in many cellular processes such as glycogen metabolism, cytoskeletal control, neurotransmission, phosphate activity and control of NAD^+/$NADP^+$ Calmodulin binds proteins principally through their interactions with hydrophobic sites on its surface. These sites are exposed after a conformational change induced by the action of Ca^{2+} on separate Ca^{2+}-binding sites. The binding of enzymes may be enhanced if the enzyme substrate is present and enzyme-substratecalmoldulin- Ca^{2+} complexes are particularly stable.

Sepharose™

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Table 1. Medium characteristics.

Ligand density:	0.9–1.3 mg calmodulin/ml drained medium
Binding capacity:	No data available
Bead structure:	4% agarose
Bead size range:	45–164 µm
Mean particle size:	90 µm
Max. linear flow rate*:	75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH stability**	
Long term:	4–9
Short term:	4–9
Chemical stability:	Stable to all commonly used aqueous solutions
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.

* Linear flow rate= $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

** The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration and cleaning procedures.

1. Preparing the medium

Calmodulin Sepharose 4B is supplied pre-swollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replace it with binding buffer in a ratio of 75% settled medium to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

2. Packing Sepharose 4B

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the medium slurry.

3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the 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.
5. Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
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, see Table 1, is typically employed during packing.

Note: Do not exceed 75% of the packing flow rate in subsequent chromatographic procedures.

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

3. Using an adaptor

Adaptors should be fitted as follows:

1. After the medium has 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 buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.
4. 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 on the column should be turned in all directions during this procedure to ensure that air is removed.

5. Lock the adaptor 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 adaptor on the medium surface as necessary.

The column is now equilibrated and ready for use.

4. Binding

Calmodulin binds proteins principally through hydrophobic interactions. Hydrophobic sites are exposed by the use of a low (1–2 mM) concentration of Ca^{2+} in the buffer. However, it has been shown that some non-specific ionic interactions can occur. Use of low concentrations of salt, 0.05–0.20 M NaCl, will therefore promote biding to the ligand whilst eliminating any non-specific binding. A common buffer used for binding is 50 mM Tris-HCl, pH 7.5, 0.05–2M NaCl, 2 mM $CaCl_2$.

5. Elution

Proteins are eluted from the medium by stripping the calmodulin of Ca^{2+}, thereby reversing the conformational change which expose the protein binding sites. A chelating agent such as EGTA (2mM) is ideal. EDTA can be used but is less efficient. 50 mM Tris-HCl, pH 7.5, 0.05–2 M NaCl, 2 mM EGTA can be used for elution.

A stronger binding to the immobilised calmodulin may be seen if the enzyme substrate is present.

Since the calmodulin binding sites on proteins appear to be very susceptible to protease action, increased yields of enzyme are obtained if proteases are removed as quickly as possible from the sample to be applied.

Removal of free calmodulin from the sample before it is applied to the column is recommended to increase the yield of enzymes to be purified. This can be done by hydrophobic interaction chromatography in the presence of Ca^{2+} on HiTrap™ Phenyl FF (high sub) or by ion exchange chromatography on HiTrap Q FF.

6. Regeneration

After use, Calmodulin Sepharose 4B should be regenerated before it is re-equilibrated with binding buffer. Recommended regeneration procedures are as follows:

- Wash with 3 column volumes of 0.1 M ammonium carbonate buffer pH 8.6 containing 2 mM EGTA.
- Wash with 3 column volumes of 1 M NaCl containing 2 mM $CaCl_2$.
- Wash with 3 column volumes of 0.1 M sodium acetate buffer pH 4.4 containing 2 mM $CaCl_2$.
- Wash with binding buffer containing 1–2 mM $CaCl_2$

alternatively

- Wash with 3 column volumes of 50 mM Tris-HCl, pH 7.5, containing both 2 mM EGTA and 1.0 M NaCl.
- Re-equilibrate with 3 column volumes of binding buffer containing 2 mM $CaCl_2$.

7. Cleaning

In some applications, substances such as denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the medium with a non-ionic detergent, e.g. 0.1% Triton™ X-100 at 37 °C for one minute followed by reequilibration with 3 column volumes of binding buffer.

8. Storage

Calmodulin Sepharose 4B should be stored at +4 to +8 °C in 20% ethanol. The medium must not be frozen.

9. Further information

Check www.chromatography.amershambiosciences.com for more information. Useful information is also available in the Affinity Chromatography Handbook, see ordering information.

10. Ordering information

Product	Pack size	Code No.
Calmodulin Sepharose 4B	10 ml	17-0529-01

Related Products

HiTrap Phenyl FF (high sub)	5 × 1 ml	17-1355-01
HiTrap Q FF	5 × 1 ml	17-5053-01

Literature

Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

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