Calmodulin Sepharose 4B

Product Information

Cat#No#	Ca-350C

Product Overview

Calmodulin Sepharose 4B affinity resin for purification of proteins with affinity for calmodulin, a highly conserved regulatory protein involved in many cellular processes in eukaryotic cells.
For single-step purification of native calmodulin-binding proteins.
Suitable for tandem affinity purification (TAP) of protein complexes.
Purification of calmodulin-regulated proteins from all eukaryotic cells.

Description

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.

Medium Preparation

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.

Ligand Coupling Method

Cyanogen bromide activation

Matrix

Agarose, 4%

Particle Size

45 μm-165 μm

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CREATIVE BIOMART®
Chromatography

Calmodulin Sepharose 4B

Average particle size
~90 µm
Ligand
Calmodulin
Recommended flow rate
< 75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
Chemical stability
Stable to commonly used aqueous solutions
Physical stability
Negligible volume variation due to changes in pH or ionic strength.
pH working range
4–9
CIP stability
4–9
Storage
2 to 8°C, 20% Ethano
Binding buffer
50 mM Tris-HCl, pH 7.5, 0.05 to 2M NaCl, 2 mM CaCl2
Elution buffer
50 mM Tris-HCl, pH 7.5, 0.05 to 2 M NaCl, 2 mM EGTA
Binding
Calmodulin binds proteins principally through hydrophobic interactions. Hydrophobic sites are exposed by the use of a low (1 to 2 mM) concentration of Ca2+ in the buffer. However, it has been shown that some non-specific ionic interactions can occur. Use of low concentrations of salt, 0.05 to 0.20 M NaCl, will therefore promote biding to the ligand whilst eliminating any nonspecific binding. A common buffer used for binding is

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50 mM Tris-HCl, pH 7.5, 0.05 to 2M NaCl, 2 mM CaCl2 .

Elution

Proteins are eluted from the medium by stripping the calmodulin of Ca2+, 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 to 2 M NaCl, 2 mM EGTA can be used for elution.

Regeneration

After use, Calmodulin Sepharose 4B should be regenerated before it is reequilibrated 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 CaCl2 . 71707600 AE 7.
Wash with 3 column volumes of 0.1 M sodium acetate buffer pH 4.4 containing 2 mM CaCl2.
Wash with binding buffer containing 1 to 2 mM CaCl2 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 CaCl2.

Cleaning-in-place

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 nonionic detergent, at 37°C for one minute followed by reequilibration with 3 column volumes of binding buffer.

Pack size

10 mL

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