Calmodulin Resin

INTRODUCTION

Calmodulin is immobilized on 4% agarose using the cyanogen bromide method to make Calmodulin Resin. This resin is ideal for the purification of calmodulin binding proteins that are involved in many biological pathways, including glycogen metabolism, neurotransmission and cytoskeletal control. In addition, a growing use is the isolation of recombinant proteins that are fused to the calmodulin-binding peptide (CBP).

ITEMS SUPPLIED Cat. # 786-282

DESCRIPTION	Size
Calmodulin Resin*	10ml resin

**Calmodulin Resin is supplied 20ml as 50% slurry in 20% ethanol*

STORAGE CONDITIONS

Shipped at ambient temperature. Store refrigerated at 4°C. **DO NOT FREEZE**. This product is stable for 1 year at 4°C.

SPECIFICATIONS

Ligand Density: 0.9-1.2mg calmodulin/ml resin
Binding capacity: 1-3mg/ml (approx.)
Bead Structure: 4% agarose
Bead Size: 50-160µm

ITEMS NEEDED BUT NOT SUPPLIED

Disposable columns
Binding Buffer: 50mM Tris, pH 7.5, 0.05-0.2M NaCl, 2mM $CaCl_2$
Elution Buffer: 50mM Tris, pH 7.5, 0.05-0.2M NaCl, 2mM EGTA

> ***NOTE:*** *For the binding buffer, the hydrophobic sites are exposed in the presence of Ca^{2+}, however in some cases there is an increase in non-specific binding, which can be eliminated by the presence of low salt concentrations (0.05-2.0M NaCl). EDTA may be used in place of EGTA in the elution buffer, however it is less efficient.*

Regeneration reagents: 0.1M $NaHCO_3$, pH 8.6, 2mM $CaCl_2$; 1M NaCl, 2mM $CaCl_2$; 0.1M acetate buffer, pH 4.4, 2mM $CaCl_2$; 20% ethanol

PREPARATION BEFORE USE

Sample preparation: Common lysis buffers are compatible with the resin, but must contain 2mM $CaCl_2$. The following list is the maximum compatible levels of some common reagents: 50-300mM NaCl/ KCl/ NH_3SO_4, 5mM DTT, 10mM β-mercaptoethanol, 0.1% TX-100/ NP-40. Avoid EDTA and EGTA.

PROTOCOL

1) Add an appropriate amount of calmodulin resin to a suitable tube (suitable to hold 7 columns volumes (CV)). Allow resin to settle and carefully decant the storage buffer.

2) Equilibration Step: Resuspend the resin in 5CV of binding buffer and allow resin to settle. Decant the supernatant and repeat step 2 once. Finally add an equal volume of binding buffer to the resin.

Column Method

NOTE: *Reaction can be performed at 4°C or room temperature. Ensure all reagents and components are at the same temperature.*

3) Add 10% volume of binding buffer to the column and pipette in the desired amount of calmodulin resin. Allow the column to drain.

4) Gently load an appropriate volume of sample. Allow column to drain under gravity.

5) Wash Step: Wash the column with 10CV of binding buffer to remove unbound material.

5) Elution Step: Elute the bound proteins in a stepwise manner with 0.5-2ml aliquots of elution buffer.

6) Identify the CBP-tagged protein fractions using a suitable protein assay (NI-Protein Assay Cat. # 786-005).

Batch Binding Method

1) Add the equilibrated Calmodulin Resin directly to the sample lysate and allow binding for several hours to overnight with mechanical rotation at 4°C.

2) After binding, pour the resin into a column and wash with at least 10CV of binding buffer, or until there is no protein in the flow-through (measure absorbance at 280nm or use a protein assay (NI-Protein Assay Cat. # 786-005))

3) Elute the protein with 10CV of elution buffer in a stepwise manner with 0.5-2ml aliquots of elution buffer.

4) Identify the CBP-tagged protein fractions using a suitable protein assay (NI-Protein Assay Cat. # 786-005).

Column Regeneration

1) Wash the resin with 3CV of 0.1M $NaHCO_3$, pH 8.6 containing 2mM EGTA.

2) Wash with 3CV of 1M NaCl containing 2mM $CaCl_2$.

3) Wash with 3CV of 0.1M acetate buffer, pH 4.4 containing 2mM $CaCl_2$.

4) Wash with binding buffer and store washed resin at 4°C in 20% ethanol.

NOTE: *Do not regenerate resin more than 3 times.*

RELATED PRODUCTS

1. Nickel Chelating Resin (Cat. #786-281)*: Immobilized metal affinity chromatography (IMAC) resin utilizing nickel (Ni^{2+}) for the purification of 6x histidine tagged proteins.*

2. HOOK™ Agarose Coupling Kit (Sulfhydryl reactive) (Cat. #786-064): *For the coupling of peptides and proteins to agarose through their sulfhydryl groups.*

3. HOOK™ Agarose (Amine reactive) (Cat.# 786-066): *Agarose for the coupling of peptides and proteins via their primary amine residues.*

4. Empty columns (Cat.# 786-169): *Empty columns for the generation of small affinity columns.*

5. Tube-O-Dialyzer™: *Allows dialysis of small samples without having to take the sample out of the tube thus eliminates its loss (Medi & Micro size available with 1kDa, 4kDa, 8kDa, 15kDa & 50kDa MW cut off limits).*

Note: For other related products, visit our web site at **www.GBiosciences.com** or contact us.