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A Geno Technology, Inc. (USA) brand name

Calmodulin Resin Kit

(Cat. #786-552)

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).

ITEM(S) SUPPLIED (Cat. # 786-552)

Description	Size
Calmodulin Resin*	10ml
Calmodulin Binding/Wash Buffer	100ml
Calmodulin Elution Buffer	100ml
Column, 5ml	5
Spin Column, 1ml	5
Rubber Stoppers (Small)	10
Caps, Snap	5

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

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the Calmodulin Resin refrigerated at 4°C **(DO NOT FREEZE)** and all other components at room temperature. The kit components are stable for 1 year when stored and used as recommended.

SPECIFICATIONS

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

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% Triton® X-100/ Nonidet® P-40. Avoid EDTA and EGTA.

PROTOCOL

1. Add an appropriate amount of Calmodulin Resin into the column of your choice. Allow the resin to settle and the storage buffer to drain by gravity flow into a waste container.
2. Equilibration Step: Resuspend the resin in 5column volume (CV) of binding buffer and allow the resin to settle. Allow the buffer to drain into a waste container by gravity flow.
   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 Column Volume of Calmodulin Binding/Wash Buffer to the column and allow the liquid to drain under gravity.
4. Gently load an appropriate volume of sample and allow the column to drain under gravity.
5. Wash Step: Wash the column with 10CV of binding buffer to remove unbound material.
6. Elution Step: Elute the bound proteins in a stepwise manner with 0.5-2ml aliquots of elution buffer.
7. Identify the CBP-tagged protein fractions using a suitable protein assay *(e.g. G-Biosciences 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).*

RESIN REGENERATION

Resin Regeneration Reagents (*Not supplied with the kit*):

(i) 0.1M $NaHCO_3$, pH 8.6 and 2mM $CaCl_2$
(ii) 1M NaCl and 2mM $CaCl_2$
(iii) 0.1M acetate buffer, pH 4.4 and 2mM $CaCl_2$;
(iv) 20% ethanol

For resin regeneration, follow the steps as under:

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

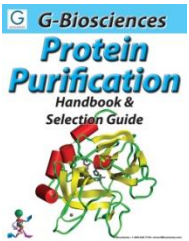
2) Wash with 3CV of 1M NaCl containing 2mM $CaCl_2$.

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

Download our Protein Purification Handbook.



http://info2.gbiosciences.com/complete-protein-labeling-conjugation-handbook
For other related products, visit our website at www.GBiosciences.com or contact us.

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