FOR RESEARCH USE ONLY!

Calmodulin-Sepharose Beads

03/15

Store at 4°C. Do not freeze

Cat. No.: 7934-1	**1ml**
Cat. No.: 7934-5	**5 ml**
Cat. No.: 7934-10	**10 ml**

FORMULATION: Supplied as 50% aqueous slurry in 20 % Ethanol.
LIGAND DENSITY: ~ 1.5 mg Calmodulin protein per ml of drained resin/beads.
PREPARATION: Calmodulin-Sepharose Beads are prepared by covalently coupling recombinant human calmodulin (Cat # 7838) to 6% cross linked Sepharose beads.

INTRODUCTION: Calmodulin is a highly conserved regulatory eukaryotic protein involved in various cellular processes such as glycogen metabolism, cytoskeletal control, neurotransmission, phosphate activity and control of NAD/NADP. The diverse functions of calmodulin rely on its ability to interact with, and regulate the activities of large number of its target proteins/enzymes. Calmodulin binds proteins usually through its hydrophobic sites, which are exposed due to conformational changes in the presence of Ca^{2+}. However, some target proteins interact with Calmodulin in a calcium-independent manner. Calmodulin-Sepharose beads allow for specific enrichment of known or unknown calmodulin-binding proteins from biological samples. In addition, Calmodulin-Sepharose beads provide a convenient tool for affinity purification of calmodulin binding peptide (CBP) tagged recombinant proteins. Elution of CBP tagged proteins could be achieved by simply adding chelation ligand / removing Ca^{2+}.

APPLICATIONS:

- Single-step purification of native calmodulin-binding proteins.
- Tandem affinity purification (TAP) of protein complexes.
- Purification of CBP tagged proteins.
- Purification of calmodulin-regulated proteins from all eukaryotic cells.

RECOMMENDED BUFFERS:

Lysis / Binding / Wash buffer:

50 mM Tris Buffer, pH 7.4, 50-200 mM NaCl, 2 mM $CaCl_2$

- Avoid EDTA or EGTA in the buffers.
- Beads compatible with common reagents: 5 mM DTT, 10 mM β-mercaptoethanol, 0.1% TX-100/NP-40, and protease inhibitors.
- Increase the NaCl concentration for stringent washes if needed

Elution buffer:

Tris buffer pH 7.4, NaCl 50-200 mM, EGTA 5 mM

RECOMMENDED PROTOCOLS:

Batch Purification

1. Prepare samples in a Ca^{2+} containing buffer.
2. Wash beads with dH_2O, then equilibrate with binding buffer containing Ca^{2+} (For e.g., 50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$).
3. Add the equilibrated beads directly to the sample lysate and incubate for several hours to overnight with gentle mixing (4°C).
4. Pour the beads-lysate mixture into a column, and wash with Ca^{2+} containing binding buffer, until no protein can be detected in the flow-through.
5. Elute with EGTA elution buffer (for e.g., 50 mM Tris, pH 7.4, 150 mM NaCl and 5 mM EGTA).
6. For Ca^{2+} -independent calmodulin binding proteins, glycine buffer (pH 2.0), urea elution buffer or boiling in SDS-loading buffer can be used.

Column (Gravity) purification

1. Prepare samples in a Ca^{2+} containing buffer.
2. Fill a column with the desired amount of Calmodulin-Sepharose beads and allow the beads to settle.
3. Wash the column with at least 5 column volumes of dH_2O.
4. Then wash the column with 5 column volumes of Ca^{2+} containing binding buffer.
5. Load the sample solution onto the washed column.
6. Collect the solution and repeat step 5 & 6 several times if necessary.
7. Wash the column with 5-10 column volumes of the binding buffer, until no protein can be detected in the flow-through, (increase the NaCl concentration if needed)
8. Add EDTA containing elution buffer to elute the bound proteins. Collect the eluents and store at appropriate temperatures.
9. For Ca^{2+} -independent calmodulin binding proteins, glycine buffer (pH 2.0) or urea elution buffer can be used.

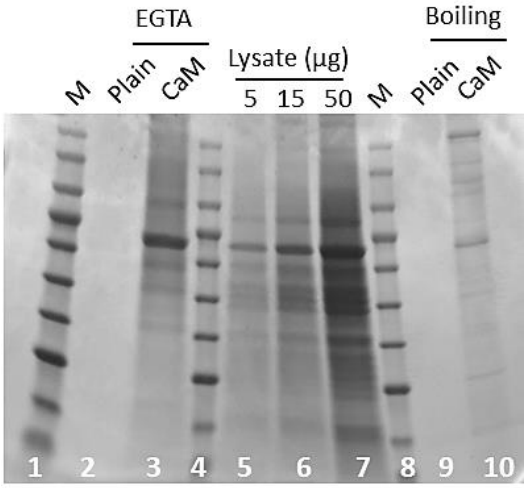

Figure: **Enrichment of Calmodulin Binding Proteins (Calcium-dependent or -independent) using the Calmodulin-Sepharose:** 293F cells were lysed with Lysis Buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1% Triton X-100, 2 mM $CaCl_2$ and protease inhibitors). Clear lysate was collected after centrifugation at 16,000 g for 30 min at 4 ˚C. Calmodulin-Sepharose beads (CaM) and Plain beads (Plain) were washed with dH_2O, and equilibrated with Wash Buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$). Approximately 25 mg clarified cell lysate (5.8 mg/ml) were incubated with 1 ml of Plain beads or CaM beads at 4˚C for 2 hours. After incubation, beads were extensively washed with Lysis Buffer, Wash Buffer, and finally with 500 mM NaCl in Wash Buffer. Beads were first eluted with 5 mM EGTA. Equal proportions (20%) of concentrated EDTA eluent were resolved on SDS-PAGE gel (EGTA, 2, 3). The proteins remaining bound to beads were eluted by boiling in 1x SDS loading buffer, and equal proportion of samples (5%) were used for SDS-PAGE (Boiling, 9, 10). Elution of proteins by EGTA and boiling is suggestive of Ca^{2+} -dependent interaction (2, 3) and Ca^{2+} -independent interaction (9, 10) respectively. This clearly indicates that large populations of proteins are captured by CaM beads (3 & 10), relative to the plain beads (2 & 9). Proteins eluted from CaM beads clearly display a distinct pattern from those in cell lysate (lane 5, 6, 7), supporting the enrichment of specific populations of proteins from lysates.

RELATED PRODUCTS:

- Calmodulin, human recombinant **(Cat No. 7838-500)**
- Calmodulin, Bovine Brain **(Cat. No. 7291-500, -1000)**
- Active CAMK1b **(Cat. No. 7729-5)**
- Active CAMK1d **(Cat. No. 7713-5)**
- Active CAMK1G **(Cat. No. 7736-5)**
- Active CAMK4 **(Cat. No. 7740-5)**
- CaMKII Antibody **(Cat. No. 3383-100)**
- Phospho-CaMKII Antibody **(Cat. No. 3384-100)**
- Phospho-MARCKS Antibody **(Cat. No. 3650-100)**

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