

## Purification of Calmodulin from Bovine Brain

References: Watterson *et al.* (1980) *J Biol Chem* 255:962-75.

Charbonneau *et al.* (1979) *Biochem Biophys Res Commun* 90:1039-47.

### Extraction of Calmodulin

#### Solutions:

1. 1 N NH<sub>4</sub>OH stored at room temperature.
2. 1 N sulfuric acid in 50% AmSO<sub>4</sub> stored at room temperature.
3. 2 M Tris Base stored at 4°C.

The following buffers are made up the day before and kept at 4°C:

4. Homogenizing buffer - 4 liters of 50 mM MOPS, pH 7.0, 1 mM 2-mercaptoethanol, 1 mM EDTA.
5. Dialysis buffer - 6.8 liters of 10 mM Tris, pH 7.5, 50 mM NaCl.

#### Procedure (0-4°C):

1. About 1 kg of bovine brain (PelFreeze, frozen) is homogenized in 2 volumes of homogenizing buffer (50 mM MOPS, pH 7.0, 1.0 mM 2-ME, 1.0 mM EDTA) for 90 sec in a large Waring blender.
2. The homogenate is centrifuged at 10,000 x g for 1 hr. The supernatant fraction is decanted and stored at 0-4°C.
3. The pellets are placed in the blender with the same volume of homogenizing buffer used in step 1 and homogenized for 90 sec.
4. Centrifuge the homogenate for 1 hr at 10,000 x g and combine the supernatant with the first supernatant. Discard the pellet.
5. Ammonium Sulfate Precipitation - Add solid ammonium sulfate (313 gm/l) slowly to the supernatant to 50% saturation, adjusting the pH to 7.0 with 1 N NH<sub>4</sub>OH. Stir for 1 hr.
6. Centrifuge for 30 min at 10,000 x g. Save the supernatant, filtering it through glass wool. Discard the pellet.
7. Isoelectric Precipitation - Adjust the pH of the sup to 4.0 with 1 N sulfuric acid in 50% AmSO<sub>4</sub> solution. Stir for 1 hr.
8. Centrifuge for 30 minutes at 10,000 x g and discard supernatant solution.
9. The pellet is resuspended in 30-50 ml of dialysis buffer (10 mM Tris, pH 7.5, 50 mM NaCl). The pH is adjusted with 1 M Tris base to pH 7.0 (see note 4).
10. The solution is dialyzed against 4 liters of 10 mM Tris, pH 7.5, 50 mM NaCl with

several changes.

Notes:

1. If frozen brains are used, they are thawed overnight at 4°C.
2. The blender and centrifuge bottles are pre-chilled by placing them in the cold room the night before the prep.
3. All the above steps are done at 4°C.
4. This pellet (step 9) will slowly go into solution as the pH is adjusted to 7.0.

### DE-52

Solutions:

1. 50 mM MOPS, pH 7.0, 0.1 M KCl - 1 liter column buffer.
2. 50 mM MOPS, pH 7.0, 0.1 M KCl - 1 liter elution buffer.
3. 50 mM MOPS, pH 7.0, 0.4 M KCl - 1 liter elution buffer.

Procedure (0-4°C):

1. Pour a 100 ml DE-52 column equilibrated with 50 mM MOPS, pH 7.0, 0.1 M KCl.
2. Centrifuge the dialyzed sample at 50,000 x g for 30 min and apply supernatant fraction to equilibrated column.
3. Wash the column with 50 mM MOPS, pH 7.0, 0.1 M KCl until no more protein elutes (using Coomassie assay).
4. Elute the calmodulin with a 2 liter gradient from 0.1 M KCl to 0.4 M KCl with 50 mM MOPS, pH 7.0.
5. Collect 20 ml fractions and measure protein concentration conductivity and CM activity of the fractions.
6. Pool the peak containing calmodulin activity for the phenothiazine column.

Notes:

The activity will come off in a rather broad peak around 0.2-0.25 M KCl.