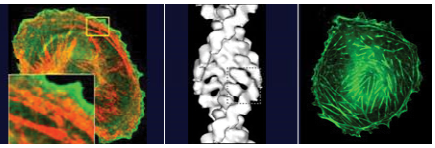

Calmodulin Preparation

Adapted from, Putkey et al. (1985) J. Biol. Chem. 260:4704-4712

BUFFERS and COLUMNS:

Lysis Buffer:
50 mM Tris, pH 7.5
2 mM EDTA
5 mM DTT
100 mM NaCl
1 mM PMSF
Protease inhibitor cocktail

EDTA Buffer:
50 mM Tris, pH 7.5
2 mM EDTA
5 mM DTT

EDTA Buffer with NaCl:
50 mM Tris, pH 7.5
2 mM EDTA
5 mM DTT
100 mM NaCl

Calcium Buffer:
50 mM Tris, pH 7.5
1 mM $CaCl_2$
5 mM DTT

Calcium Buffer with NaCl:
50 mM Tris, pH 7.5
1 mM $CaCl_2$
5 mM DTT
100 mM NaCl

$CaCl_2$ Stock:
1 M $CaCl_2$

Dialysis Buffer:
10 mM Tris, pH 7.5
50 mM KCl
1 mM EGTA
1 mM DTT

Urea Stock:
6 M Urea

Columns
Two phenyl-sepharose CL-4B columns (10 mL each). Columns can be cleaned at room temperature, but should be equilibrated, loaded, and eluted at 4 °C.

PREPARATION:

1. Grow and induce 2 x 500 mL bacteria to express calmodulin. Spin down and freeze.

2. Wash each column with 20 mL 6 M urea followed by 20 mL Millipore H_2O. Equilibrate in the appropriate buffer:
    a. Column-A: 50 mL EDTA Buffer with NaCl
    b. Column-B: 50 mL Calcium Buffer with NaCl

3. Resuspend each pellet in 15 mL Lysis Buffer. Keep on ice.

4. Combine pellets and sonicate to lyse cells. Keep on ice.

5. Spin 15,000 RPM in SS-34 rotor, 30 min, 4 °C.

6. Load supernatant onto Column-A (which was equilibrated with EDTA Buffer with NaCl), and wash with an additional 15 mL of EDTA buffer with NaCl. SAVE ELUATE.

7. Add $CaCl_2$ stock to eluate to a final concentration of 7 mM $CaCl_2$. (This results in a free calcium concentration of ~ 5 mM).

8. Load eluate on to Column-B and wash with 30 mL Calcium Buffer.

9. Wash column with 30 mL Calcium Buffer with NaCl.

10. Wash column with 30 mL Calcium Buffer.

11. Eluate calmodulin from column with 45 mL EDTA Buffer, collecting 3 mL fractions.

12. Measure OD_{275} and run samples on 15% SDS-PAGE to determine which fractions to pool.

13. Concentrate to ~ 3 mL using spin-concentrator and dialyze vs. Dialysis Buffer.

14. Further purify via Mono-Q, if required.

15. Determine concentration by absorbance ($\varepsilon_{275} = 3300\ M^{-1}cm^{-1}$). Note, calmodulin does not contain tryptophan. Also, colorimetric dyes (i.e., Bradford Reagent) tend to underestimate the true protein concentration.