Human Cofilin Purification

Make an extraction buffer (2 L):

20 mM MOPS pH 7.0
1 mM NaN_3
0.5 mM EDTA
50 mM NaCl
2 mM DTT (add right before use)
0.5 mM PMSF (add right before use)

Make columns:

- First column – DE52
- Second column – SP sepharose
- Both columns connected sequentially (first DE52, then Sepharose)
- Connect to the pump and run 150 mL (10-15 column volumes) of the extraction buffer through both columns at the elution rate of ~1.5 mL/min.

Lyse the cells:

- Add cold (4^{o}C) extraction buffer (with DTT and PMSF added + 1 mM PMSF + 1:500 protease inhibitors + 5 mM benzamidine) to the frozen cell pellet up to 40 mL. Mix thoroughly (homogenize) until it's a nice suspension on ice.
- Use the French press to mechanically disrupt the cells (do not exceed 1200 PSI). Keep on ice at all times.
- Spin the lysate for 30 min at 20,000 rpm at 4^{o}C.
- Collect and filter the supernatant (save the pellet).

Run the columns:

1. Run the filtered supernatant through both columns connected to each other (first DE52, then Sepharose). Collect the flow-through.
2. Wash the connected columns with the extraction buffer until no more protein is present in the washing fraction (verified by Bradford Assay). Collect the wash fraction.
3. Disconnect the columns. Connect the pump directly to the Sepharose column (where cofilin is supposed to be bound).
4. Set up the gradient maker: add 75 mL of 700 mM NaCl (outer cylinder) and 75 mL of 50 mM NaCl (inner cylinder). Put a stir bar to the inner cylinder. Keep closed until ready to run.
5. Set up the fraction collector, set the timer on the fraction collector and start eluting with salt gradient from the opened and stirring gradient maker. Collect all fractions.
6. Analyze fractions on 15% SDS-gel (run every other fraction).

7. Combine fractions that have the desired purified protein.
8. Use a concentrator to concentrate the protein further to about 2-3 mL (MWCO <3000kDa).
9. Set up a dialysis (wear gloves). Dialysis buffer: (2 L)
   10 mM MOPS pH 7
   25 mM NaCl.
   0.1 mM PMSF
10. Measure the concentration (A_{280}).

    Human cofilin 1 – 18.5 kDa; pI=8.22

    Extinction coefficients:
    Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.
    Ext. coefficient 14690
    Abs 0.1% (=1 g/l) 0.794, assuming all pairs of Cys residues form cystines
    Ext. coefficient 14440
    Abs 0.1% (=1 g/l) 0.780, assuming all Cys residues are reduced

    Human cofilin 2 – 18.7 kDa; pI=7.66

    Extinction coefficients:
    Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.
    Ext. coefficient 18575
    Abs 0.1% (=1 g/l) 0.991, assuming all pairs of Cys residues form cystines
    Ext. coefficient 18450
    Abs 0.1% (=1 g/l) 0.985, assuming all Cys residues are reduced

11. Aliquot and save the protein at -80^{o}C.