# Preparation of Brain Profilin and Profilin-Actin Complexes

## Materials

1. 1 mM EDTA, 0.1 mM ATP, 0.1 mM PMSF (from stock of 100 mM in 95% alcohol, highly toxic), 0.5 mM DTT, 10 mM imidazole-HCl, pH 7.0, $4^oC$. Need 3 ml per gram wet tissue.

2. 100 mM glycine, 100 mM NaCl, 1 mM DTT, 10 mM Tris-HCl, pH 7.8, $4^oC$. Need 15 liters. Make 10x, dilute and titrate pH.

3. PBS solution A, 1000-2000 ml, $4^oC$.

4. Poly-L-proline affinity column, 2.5x15 cm. Conjugate poly-L-proline (m.w. 10,000, Sigma P-2129) to CNBr-Sepharose. Pre-equilibrate with buffer 2 at $4^oC$. The column can be used repeatedly for many preparations. However, it should be cycled batchwise through acidic and basic buffers when there is a noticable reduction in flow rate. See steps 12-14 in the procedure for conjugating proteins to sepharose.

5. Waring blender, prechilled.

6. Fraction collector and UV monitor.

7. 3 M urea in buffer 2, 400 ml.

8. 8 M urea in buffer 2, 400 ml.

9. Type 35 rotor and bottles.

## Procedure

1. Rinse out blood with PBS and trim brain in the cold room. Remove blood vessels, connective tissue, and white lipids. Trimmed tissue can be stored at $-80^oC$. When using frozen tissue, let it thaw in cold PBS over 1-2 hr at room temperature. Weigh the cleaned tissue. Start with 500-700 g.

2. Homogenize in 3 volumes of buffer 1, using several short bursts (~5 sec) in a Waring blender at a medium to high speed in the cold room.

3. Centrifuge in a GSA rotor at 11,000 rpm, $4^oC$ for 1 hr.

4. Collect supernatant and centrifuge in a Type 35 rotor at 33,000 rpm, $4^oC$ for 3 hr.

5. Load supernatant into the poly-L-proline column overnight. The maximum operating pressure for Sepharose 4B is 80 cm $H_2O$.

6. Wash the column with 1200 ml buffer 2.

7. Wash with 3 M urea in buffer 2.

8. Elute with 8 M urea in buffer 2. Set sensitivity of UV monitor at 0.5. The column can be regenrated after extensive wash with buffer 2.

9. Collect peak fractions. Dialyze against 4 liters of buffer 2 at $4^oC$, change buffer 2 times over a 36 hr period.

10. Concentrate profilin in a colloidin bag with m.w. 10,000 cutoff, by either vacuum dialysis or aquacide II. Should yield ~0.5 mg profilin from 100 g wet tissue. Store in liquid nitrogen.

## Reference

**D.A.Kaiser, P.J.Goldschmidt-Clermont, B.A.Levine and T.D.Pollard** (1989) Characterization of renatured profilin purified by urea elution from poly-L-proline agarose columns. *Cell Motil. Cytoskeleton* 14:251-262.

# Preparation of Profilin-Actin Complexes

## Materials

1. See profilin preparation, items 1, 3, 4.

2. Buffer 2 as for profilin preparation, but without DTT.

3. 30% DMSO in buffer 2 without DTT, 400 ml.

4. Centriprep-30.

## Procedure

1. Load the poly-L-proline column as for the preparation of profilin.

2. Wash the column with 1200 ml buffer 2 without DTT.

3. Elute the column with 30% DMSO in buffer 2. The column cannot be regenerated after use with DMSO.

4. Collect peak fractions. Dialyze against 4 liters of buffer 2 at 4°C, change buffer 2 times over a 36 hr period. DTT is added for the second and third buffer .

5. Concentrate with Centriprep-30. Free profilin should go through the filter. Store in liquid nitrogen.

# Reference

**M.Rozycki, C.E.Schutt and U.Lindberg** (1991) Affinity chromatography-based purification of profilin:actin. *Methods Enzymol.* 196:100-118.

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