Preparation of Protein Extract for F-Actin affinity chromatography

(Adapted from Miller et al. (1989) JCB 109: 2963-2975 as in Sisson et al. (2000) JCB 151(4):905-917)

All steps should be done at 4ºC

1. Homogenize dechorionated embryos in 10 volumes of E-buffer. Add PMSF to 1 mM just before homogenizing. Use a motor-driven Teflon-tipped pestle and homogenize with 5 full strokes.

2. Spin the homogenate at 10,000 x g for 20' at 4ºC.

3. Place a funnel into a graduated cylinder and line the funnel with sterile cheesecloth.

4. Decant the supernatant onto the cheesecloth and record the volume of the filtered supernatant.

5. Adjust the final concentration of Hepes to 50 mM and add DTT to 2 mM, gently mix the supernatant, then spin it at 100,000 x g for 1 hour at 4ºC.

6. Finally, collect the supernatant (S100) with a pipette avoiding the lipid layer.

E-buffer
5 mM Hepes pH 7.5
0.5 mM EDTA
0.5 mM EGTA
0.05% NP-40
Protease Inhibitor Cocktail (1:100)

Stock Protease Inhibitor Cocktail
0.04 g Benzamidine HCl (10 μM)
0.003 g Phenanthroline (1.2 μg/ml)
0.025 g Aprotinin (10 μg/ml)
0.025 g Leupeptin (10 μg/ml)
0.025 g Pepstatin A (10 μg/ml)

Resuspend the indicated amounts of each of the protease inhibitors in 95% EtOH and store at –20ºC. Dissolve the inhibitors in the EtOH at 37ºC for a short time prior to use. Note that there will always be some undissolved material. Obtain inhibitors from Sigma. Final concentrations, after a 1:100 dilution in E-buffer, are indicated in parentheses above.