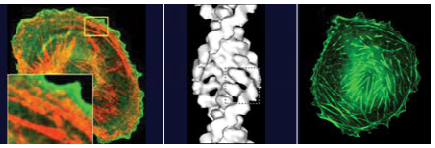

Preparation of Actin from Rabbit Skeletal Acetone Powder

(Modified from Spudich and Watt, JBC 246:4866 (1971))

Buffer A:	Stock	1 L	100 ml of 100x
2.0 mM Tris (pH 8.0 at 25° C)	1.0 M	2 ml	20 ml
0.2 mM ATP	0.1 M	2 ml	20 ml
0.5 mM DTT	dry	77 mg	770 mg
0.1 mM $CaCl_2$	1.0 M	0.1 ml	omit from 100x
1.0 mM NaAzide	1.0 M	1 ml	10 ml (or 0.65 g)

Before you start: Make sure the centrifuges are available and the rotors are cold. Turn on the Beckman ultracentrifuge, start the vacuum, and set the temperature to 4° C. **If you are making Buffer A from 100x stock, please remember to add $CaCl_2$.**

1. Mix 20 ml Buffer-A with each gram of muscle acetone powder and extract with stirring at 0° C (i.e., on ice) for 30 minutes. Yield is about 20 mg per gram.

2. Centrifuge for 30 minutes in SS-34 rotor in the RC5B centrifuge at 16,000 RPM at 4° C.

3. Filter supernatant with actin monomers through cheese cloth into a graduated cylinder and measure volume.

4. Resuspend pellets in original volume of Buffer A and repeat steps 2 and 3.

5. While stirring the combined supernatants in a beaker, make them 50 mM KCl (2.5 ml of 2 M KCl per 100 ml) and then 2 mM $MgCl_2$ (0.2 ml of 1 M $MgCl_2$ per 100 mls). This will polymerize the actin monomers. Cover and stir slowly for 1 hour at room temperature.

6. After 1 hour, make 0.8 M KCl (5.6 g of KCl per 100 ml) while stirring in the cold room. This dissociates any contaminating tropomyosin from the actin filaments.

7. After 30 min, centrifuge for 2 hours in the Ti45 rotor in the Beckman ultracentrifuge at 35,000 RPM to pellet the actin filaments.

Notes on ultracentrifuge centrifuge tubes and rotor:

- The Ti45 tubes must be > 2/3 full, or you risk them collapsing during centrifugation.
- Make sure there are no hairline cracks in the tubes that will leak when under vacuum.
- Make sure that each cap has a black-rubber O-ring.
- Make sure the rotor has its black-rubber O-rings in place.

8. Discard supernatant and gently wash off surface of pellets with Buffer A. Remove pellets with stainless steel spatula and by scraping into a small volume of Buffer A. Gently resuspend

the pellets in about 3 ml of Buffer A per original gram of acetone powder using a Dounce homogenizer. Dialyze for 2 – 3 days vs. 3 – 4 changes of Buffer A to depolymerize the actin filaments. To speed up depolymerization, you can sonicate the actin filaments gently (30 sec in a bath sonicator or 5 x 2 sec bursts with a probe sonicator.

9. Clarify the depolymerized actin solution by centrifugation in Ti45 rotor at 35,000 for 2 hours to remove aggregates. The top 2/3 of the tub contains "conventional" actin. Save the bottom 1/3. It can be spun again to remove oligomers or used as-is for pyrene labeling.

10. Gel filter on Sephcryl S-300 column in Buffer A to separate actin oligomers, capping protein and other minor contaminants from the purified actin monomers.

11. The column must be stored in 5 mM imidazole (pH 7.5), 1 mM EDTA, 1 mM azide. No ATP or DTT in storage buffer.