Preparation of Actin from Rabbit Skeletal Acetone Powder
(Modified from Spudich and Watt, JBC 246:4866 (1971))

Buffer A:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Buffer A Stock</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 mM Tris (pH 8.0 at 25° C)</td>
<td>1.0 M</td>
<td>2 ml</td>
<td>20 ml</td>
</tr>
<tr>
<td>0.2 mM ATP</td>
<td>0.1 M</td>
<td>2 ml</td>
<td>20 ml</td>
</tr>
<tr>
<td>0.5 mM DTT</td>
<td>dry</td>
<td>77 mg</td>
<td>770 mg</td>
</tr>
<tr>
<td>0.1 mM CaCl$_2$</td>
<td>1.0 M</td>
<td>0.1 ml</td>
<td>omit from 100x</td>
</tr>
<tr>
<td>1.0 mM NaAzide</td>
<td>1.0 M</td>
<td>1 ml</td>
<td>10 ml (or 0.65 g)</td>
</tr>
</tbody>
</table>

**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 ml). 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 Sephceryl 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.