ISOLATION OF ACTIN

Number:

Date:

Amount of acetone powder: ____ g

Check:

1. Extraction with buffer A **2 l** **4 l** _____

a. Buffer A:

2 mM imidazole-HCl	0.485 g	0.97 g
0.2 mM Na_2ATP	0.22 g	0.44 g
0.5 mM β-mercaptoethanol	0.078 g	0.156 g
0.2 mM $CaCl_2$	0.059 g	0.118 g
0.005% NaN_3	0.1 g	0.2 g

pH=8.0 (25°C)

b. Volume of buffer A: 20 ml/g-acetone powder

c. Extraction: stirring for 30 min, 0-0.5°C

2. Filtration _____

a. Filtration through several layers of sterile cheesecloth
and/or
[b. low-speed centrifugation at 500-1,000 g for 10-20 min.]

3. Reextraction of residue _____

a. Extraction in 20 ml/g buffer A; 10 min, 0-0.5°C
b. Combine extracts

4. Centrifugation _____

a. 10,000-20,000 g, 1 hr, 4°C (Typically, 20,000 rpm, Beckman 55.2 rotor, 30 min)
b. Pipet off (and use) supernatant

5. Polymerization _____

a. Concentrations brought to:

KCl (MW: 74.6)	50 mM
Mg^{2+}	2mM
ATP (MW: 551.1)	1 mM

b. Assemble for 2 hrs, 4°C (on ice)

6. High salt wash (Tropomyosin removal) _____

a. Add: solid KCl to 0.6 M

b. Stir gently for 0.5 hr, 4°C (30 minutes from the complete dissolution of KCl)

7. Sedimentation of F-actin _____

a. Centrifugation (in 30 ml centrif. tubes), 80,000 g, 3 hrs, 4°C
(Typically, 45,000 rpm, Beckman 55.2 rotor, 2 hours)
[b. homogenization of pellet into 150 ml wash buffer
(buffer A + 0.6 M KCl, 2 mM $MgCl_2$, 1 mM ATP)

c. Resediment F-actin 80,000 g, 3 hrs, 4°C**]**

d. Rinse F-actin pellet thoroughly with buffer A

8. Depolymerization _____

a. Allow F-actin pellet to stand in centriguge tube in ~1 ml/g-acetone powder cold buffer A for 1 hr.

b. Resuspend F-actin pellet by gentle homogenization in cold buffer A (total A-buffer volume = 3 ml/g-acetone powder)

c. Dialyze against buffer A, 4°C; change buffer A frequently (e.g., 1st, 3rd, 5th hour, plus overnight)

[for large acetone-powder quantities: divide homogenate into equal 6-ml aliquots and place them into dialysis bags 1/4 inch in diameter and 12 inches long; tie them to magnetic stirring rod in a 1-liter graduated cylinder; dialyze for 6 hrs, while stirring]

9. Clarification of G-actin _____

a. Centrifugation of dialyzed actin at 80,000 g, 3 hrs, 4°C (Typically, 45,000 rpm, Beckman 55.2 rotor, 2 hours)

10. Polymerization

a. Add:	KCl	50 mM
	$MgCl_2$	1 mM
	ATP	1 mM
	NaN_3	0.02 %

b. Store at 4°C (on ice; ensure frequent, reliable change of ice)

11. Protein concentration: $[actin](mg/ml) = \frac{OD_{280}}{1.1} \times (dilution)$

Actin cc=

*Note: a) measure against buffer A. b) start with undiluted actin; if OD is in the range of 2, then start diluting with buffer A. c) attach spect printout here.

12. Electrophoresis:

Sample preparation:

Gel loading:

1. 2. 3. 4. 5. 6. 7. 8. 9. 10.

13. Notes/Modifications: