Preparation of myosin

Based on Margossian, S.S,. & Lowey, S. *Methods Enzymol.* **85**, 55, 1982

Number:

Solutions, buffers:

A. Extraction buffer	volume: 500 ml	1000 ml	
0.3 M KCl	11.184 g	22.368 g	
0.09 M KH_2PO_4	6.124 g	12.248 g	
0.06 M K_2HPO_4	5.225 g	10.45 g	(pH 6.8)
Fresh: 0.2 mM ATP	350 ml (300 mM stock)	700 ml	
0.2 mM DTT	100 μl (1 M stock fresH0	200 μl	
0.23 mM PMSF	0.575 ml (0.2 M in EtOH)	1.15 ml	

B. 2M KCl	volume: 100 ml
2 M KCl	14.912 g
Fresh: 1 mM DTT	100 μl (1 M stock)

C. 0.5 M K-phopsphate	volume: 250 ml each
1. 0.5 M KH_2PO_4	17.01 g
2. 0.5 M K_2HPO_4	21.773 g

* Mix the two components together to adjust pH to 6.8, then add 100 μl of DTT stock (1 M)/100 ml of buffer, fresh.

[D. 0.5 M KCl-phosphate	volume: 100 ml
0.5 M KCl	3.728 g
0.05 M K-phosphate (pH 6.8)	(dilute buffer "C" 10x)
Fresh: 1 mM DTT	1 unit]

Steps: Check

1. Excise back muscles from rabbit and put on ice (about 150 g) _____
Weight of muscle:

2. Mince muscle in cold and EDTA-rinsed grinder _____
3. Extract muscle with 3 ml/g Extraction solution ("A") (about 450 ml) _____
Volume of extracting solution:

Total volume:

4. Stir vigorously for 15 min. _____
5. Dilute 1.5 times with ice cold water _____
Volume of water added:
Total volume:

6. Filter through gauze (yield about 900 ml); if necessary, _____
clarify by centrifugation
Volume of filtrate:

7. Precipitate myosin: dilute to 10x volume with ice cold water _____
(total volume = [(filtrate volume) — (water volume step 5)] * 10
Volume of water added:

*Note: Be generous with water. Sometimes the above equation falls short in producing complete myosin precipitation. In such case, increase water volume to 10x that of filtrate volume.

8. Allow to stand for 1 hour. Subsequently, decant and use supn. _____
9. Centrifuge (Typically, 9,000 rpm Beckman JA-10 rotor, 30 min) _____
Speed:

10. Disolve pellet in buffer "B" and buffer "C" _____
(total volume equal to pellet volume; B volume = C volume)
Volume of pellet (more often, weight of pellet in g):

Volume of buffers:

Total volume:

11. Dilute with ice cold water 1.5x _____
Volume of water added:
Total volume:

12. Centrifuge (Typically, 40,000 rpm, Beckman 55.2 rotor, 30 min) _____
Speed:

13. Precipitate supernatant by diluting 10x with ice cold _____
water while stirring. Continue to stir for 15 min.
Supernatant volume:
Volume of water added:

14. Centrifuge (Typically, 9,000 rpm, Beckman JA-10 rotor, 30 min) _____
Speed:

15. Dilute pellet in minimal volume of buffer "B+C" _____
Volume(weight) of pellet:

Volume of buffer B (1/3 of pellet):
Volume of buffer C [1/9 of (pellet+B)]:
Add DTT to 1 mM (from fresh 1 M stock)
Add $MgCl_2$ to 1 mM (from 1 M stock)

[16. Bring concentration of sample to 30-60 mg/ml with buffer "D" _____]
17. Centrifuge at high speed to defat sample _____
(Typically, 45,000 rpm, Beckman 55.2 rotor, 2 hours)
Speed:

18. Add 50% glycerol, aliquot into 1.5-ml Eppendorf tubes _____
19. Store at -20°C _____

Concentration of myosin: $[myosin](mg/ml) = \frac{OD_{280}}{0.53} \times (dilution)$

*Note: a) make ~100 dilution of myosin in 0.6 M KCl-AB (see in vitro assay worksheet) for cc measurements. b) attach spect printout here.

Notes/Modifications: