WORKSHEET

Papain Mg-S1 preparation

Number:

Date:

Number of myosin preparation:

Solutions, buffers:

Check:

1. BED   Volume: 100 ml   _____
    0.1 mM $NaHCO_3$ 0.02 ml stock (0.5M)
    0.1 mM EGTA   0.04 ml stock (0.25M) [20 μl 0.5 M]
    1 mM DTT   100 μl (fresh 1 M stock)

2. PMB   Volume 25 ml   _____
    25 mM imidazole-HCl (pH 7.4)   0.625 ml stock (1 M)
    100 mM NaCl   1.25 ml stock (2 M) [0.1461 g]
    5 mM $MgCl_2$ 62.5 μl stock (2 M) [125 μl 1M]
    1 mM DTT   25 μl stock (1 M)

3. E64 stock   Volume: 1 ml   _____
    E64   1 mg
    DMSO   to 1 ml

*Note: alternatively, use water-based stock. Record stock concentration.

4. Papain   Volume: 2 ml   _____
    5 mg/ml papain   0.01 g
    Papain Base Buffer   to 2 ml

5. Inhibitor buffer   Volume: 10 ml   _____
    5 mM $MgCl_2$ 25 μl stock (2 M) [50 μl 1M]
    5 μg/ml E64   50 μl (stock)
    BED   to 10 ml

6. 1.2 M KCl + 20 mM DTT/BED   Volume: 10 ml   _____
    1.2 M KCl   0.9 g
    20 mM DTT   200 μl stock (1 M)
    BED   to 10 ml

7. Papain Base Buffer (pH 7.4 with NaOH)   Volume: 100 ml
    5 mM cysteine   0.06 g
    2 mM EDTA   0.058 g [0.4 ml stock (0.5M)]

Steps: **Check**

1. Add 9 volumes of BED to 20 mg stock myosin in a centrifuge tube   _____
 (Falcon tube, with volume marks), and mix to precipitate the filaments.
Incubate on ice for >10 min.

2. Centrifuge at low speed in a swinging bucket rotor.   _____
(Typically, 1,600 rpm, old IEC, 1 hour)

3. Dissolve the pellet in equal volume of 1.2 M KCL+20 mM DTT   _____
in BED.
Pellet volume (estimate from marks on Falcon tube):

4. Add 19x volume of BED, incubate on ice for 10 min, centrifuge   _____
(Typically, 1,600 rpm, old IEC, 1 hour)

5. Resuspend pellet in PMB to a final concentration of 10-12 mg/ml,   _____
incubate for 10 min at 25ºC.
Pellet volume:
Volume of added buffer:
Myosin concentration ($A_{280} = 0.53$ cm^2/mg):

6. Add papain to 12.5 μg/ml, mix gently, incubate 7.5-10 min at 25˚C.   _____
Volume of papain stock added:
Digestion time:

7. Add greater than equal volume of inhibitor solution to stop reaction   _____
Volume of inhibitor added:

8. Incubate on ice for 1 hr.   _____

9. High-speed centrifugation.   _____
(Typically, 40,000 rpm, Beckman 50Ti, 30 min, 0 ˚C)

10. Store supernatant (typically 1.1 mg/ml) on ice.   _____
 Use in the motility assay for 3-5 days. A280=0.81 cm^2/mg.

Concentration: $[S-1](mg/ml) = \dfrac{OD_{280}}{0.81} \times (dilution)$

*Note: attach spect printout here.

SDS-PAGE:
Type of gel:
Percentage:
Sample preparation:

Standard:
Loading: lanes

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

Notes/Modifications: