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Procedure

1. Clarify G-actin (TL 100 100K rpm 30 min). This protocol is for 1 ml of 5 mg/ml actin. It can only help to scale up.
2. polymerize actin by addition of
    - KCL to 100 mM
    - MgCl2 to 1 mM
    - ATP to 1 mM
3. incubate on ice about an hour
4. remove all amines and sulphydryls by 2 sequential sedimentations of f-actin through 25 % glycerol cushion.
    - 10 mM Hepes pH 7.7
    - 1 mM ATP
    - 5 mM MgCl2
    - 100 mM KCl
    - 25% Glycerol

    (TL 100.4 80 K rpm 60 min 4 C)
5. resuspend pellets in 1 ml of above buffer without glycerol resuspend, sonicate gently and on ice to disperse filaments, and sediment for a second time through similar cushion.

    after second spin, resuspend to 1 mg/ml in above buffer. Sonicate gently and on ice to disperse filaments.

    (1 mg/ml actin =24 uM -> 5 mls contains120 nmoles)
6. add 4 mol NHS-Rhodamine/ mol actin:

    add 500 nmoles (0.26 mg) NHS-Rhodamine (MW=528)

    (make 1 mg/ml stock in dry DMSO, add 250 ul to actin while vortexing)
7. label 2 hr 4 C or 1 hr 25 C
8. quench reaction by adding Tris to 10 mM pH 8.0
9. pellet through glycerol cushion in F buffer (TL 100.4 80 K rpm 60 min 4 C)
    - 10 mM Tris pH 8
    - 1 mM ATP
    - 5 mM MgCl2
    - 100 mM KCl
    - 5 mM DTT
    - 25 % Glycerol

10. resuspend pellet in 1 ml G-buffer. first allow pellet to soften then sonicate gently
    - 10 mM Tris pH 8
    - 0.2 mM ATP
    - 0.2 mM CaCl2
    - 1 mM DTT
11. dialyse 24 hr against G buffer and spin again before freezing aliquots in LN2.
12. it may or may not be necessary to cycle the actin again. I would recommend cycling 1 aliquot to see if all of the rhodamine pellets after polymerization.