

Reagents

Biotin-XX-NHS from Molecular Probes (B-1606) ***myosin***

concentration ~75 mg/ml

Buffer A

25 mM KHepes pH7.7

40 mM KCl

2 mM MgCl₂

10 μM DTT

Buffer B

10 mM Pipes

10 mM imidazole

2 mM MgCl₂

40 mM KCl

G25 column equilibrated with

25 mM KHepes pH7.7

300 mM KCl

2 mM MgCl₂

5 mM DTT

Procedure

1. Dialyse myosin overnight vs. buffer A at 4 C.
2. Make Biotin-NHS stock in dry DMSO at 10 mM (1mg in 175 μl)
3. Mix Biotin-NHS with myosin at 0.5 mM final concentration
Should be a 3:1 molar excess over myosin
4. Incubate by rotating tube for 1 hr at RT.
5. Terminate reaction by adding glycine to 2 mM
6. Remove unincorporated biotin on G25 column in equilibration buffer
7. Dialyse ON at 4 C vs Buffer B
Should get formation of myosin filaments
8. Wash filament in buffer B 3 times by repeated pelleting and resuspension
9. pellet in eppendorf microfuge 10Krpm 30 secs.
10. Flash freeze in small aliquots and store at -80 C.

Biotinylation of Smooth Muscle Myosin