

Protocol to make biotin-actin

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Make 2 – 3 mg/ml F-actin by adding x10 KMEI
(f.50 mM KCl, 1mM MgCl₂, 1mM EGTA,
10mM Imidazole (pH7.0), 1mM NaN₃)

Maleimide-Biotin (*PIECE #21901*)
Dissolve with 950 ul DMF (f.100mM)
stored at -20 degrees.

Incubate for 1-2 hours at R.T.

Add 1M DTT (f. 5mM)

Centrifuge (100K, 45 min -1 hour at 8 degrees), then get the pellet of FA

Dissolve the pellet with G-buffer (less than a half volume of starter actin solution)
(G-buffer : 2mM Tris-HCl (pH8.0), 0.2 mM ATP, 0.5 mM DTT, 0.1 mM CaCl₂, 1mM NaN₃)

Dialysis with G-buffer at 4 degrees for 1-2 days

Centrifuge (100K, 45 min -1 hour at 2 degrees), then get the sup.of GA

Measure the concentration of biotin-actin, $A_{290}=0.0266/\mu\text{M}/\text{cm}$
(Believe the entire actin is labeled with biotin...)

Aliquot to each bottle of 10 ul, and store them at -80 degrees.

