Solution: ME buffer (f. 50uM $MgCl_2$ and 250uM EGTA), make x100 high conc.
IM buffer (f. 10 mM Imidazole (pH7.0), 50 uM $MgCl_2$)

Preparation:
100 ul ATP-G-actin
|
Add 1ul x100ME and incubate for 30 min on ice.
|
| Meantime, prepare the diluted hexokinase (f.0.38 units/ul, SIGMA #H-5625).
|
| ex) if original conc. is 676 units/mg =12.3 mg/ml, pick 25ul and remove the supernatant by centrifuge for 1min. with 14K rpm, 4 degrees. Then add 520 ul IM buffer.
|
|
Add 1 ul Glucose (0.1M, should be f. 1mM)
Add 5.4 ul Hexokinase (0.38 units/ul, should be f. 0.02units/ul)
|
Mix (use end to end mixer) for 3 hours at 4 degree (cold room). *****
|
Centrifuge 80K 55min at 4 degree (use TLA, Ultra Centrifuge Rotor from Beckman)
Get top 80% supernatant.
|
Measure the concentrations. A290=0.0266/uM/cm.

*****Tom suggests 2 hours incubation of actin with hexokinase is enough long.

Original method is from
Tom Pollard lab, Yale Univ.