Actin Nucleotide States

“ATP”-actin (AMP-PNP-actin)

- To remove free nucleotides, mix one volume of G-actin with ½ volume of Dowex-1 slurry. Incubate for 5 min on rotator. Spin down briefly, carefully collect supernatant and repeat the above procedure one more time.
- To hydrolyze remaining ATP, add 20 units/mL of hexokinase and 2 mM glucose, incubate for 30 min on ice.
- Add 0.5 mM AMP-PNP, incubate for 30 min on ice.
- During this incubation, equilibrate gel filtration spin column in nucleotide-free buffer.
- After 30 min, pass the sample through gel filtration spin column.

“ADP”-actin

- To regular G-actin add 20 units/mL of hexokinase and 2 mM glucose, incubate for 30 min on ice.

“ADP-P_i”-actin

- To regular G-actin add BeF (0.5uL from 100x stock per 50uL rxn).
- [**1xBeF**=0.2mM BeCl, 5mM NaF]