F/G Actin Binding Assay

Reference

1. Afreen, S., & Ferreira, A. (2019). Altered Cytoskeletal Composition and Delayed Neurite Elongation in tau$_{45-230}$-Expressing Hippocampal Neurons. *Neuroscience*, *412*, 1–15. https://doi.org/10.1016/j.neuroscience.2019.05.046

Kit

1. Cytoskeleton, Inc. Actin Binding Protein Biochem Kit™ Muscle Actin, Cat# BK001

Abbreviation

1. BSA: Bovine Serum Albumin

Protocol (using Method 1 of kit protocol)

F-Actin Binding Reactions:

1. Prepare 700 µL of F-actin buffer as follows: Add 630 µL of General Actin buffer and 70 µL of Actin Polymerization buffer in a centrifuge tube. Place on ice. Discard any remaining Actin Polymerization Buffer.
2. Label six centrifuge tubes (1-6) and place on ice.
   - Tube 1 - F-actin only control sample
   - Tube 2 - α-actinin only control sample
   - Tube 3 - α-actinin and F-actin positive control sample
   - Tube 4 - BSA and F-actin negative control sample
   - Tube 5 - “test protein” only sample
   - Tube 6 - “test protein” and F-actin sample
3. Pipette 40 µL of F-actin buffer into tubes 2 and 5.
4. Pipette 40 µL of F-actin stock in each of tubes 1, 3, 4, and 6.
5. Pipette 10 µL of test protein stock into tubes 5 and 6 and mix by pipetting slowly up and down three times.
6. Pipette 10 µL of α-actinin, 2 µL of 1 M Tris-HCl pH 6.5 into tubes 2 and 3. The final concentration of α-actinin is 2 µM.
7. Pipette 2 µL of BSA and 9 µL of Milli-Q water into tube 4. The final concentration of BSA is 2 µM.
8. Pipette 10 µL of the buffer the test protein stock is dissolved in into to tube 1.
9. Incubate all tubes at room temperature for 30 min.

F-actin separation:

1. Centrifuge all tubes at 150,000 x g for 1.5 h at 24°C.
2. Carefully remove the supernatants and place them into individually labeled tubes on ice.
3. Add 10 µL of 5x Laemmli reducing-sample buffer (not included) to each supernatant containing tube.

4. Resuspend the pellets in 30 µL of Milli-Q water. Pipette up and down for 2 min, leave on ice for 10 min and repeat pipetting. Add 30 µL 2x Laemmli reducing-sample buffer (not included).
5. Run 20 µL of each sample on a 4-20% SDS-gel until the dye front is at the bottom of the gel.