Sarkosyl Fractionation Assay

Cells or Recombinant Protein

References

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Solutions

1. MES Buffer (store at -20°C): 20 mM MES, pH 6.8, 80 mM NaCl, 1 mM $MgCl_2$, 2 mM EGTA, 1% Protease Inhibitor Cocktail
2. Sarkosyl Buffer (store at -20°C): 500 mM NaCl, 10% (w/v) Sucrose, 1% (w/v) N-Lauroylsarcosine Sodium Salt (Sarkosyl)

Protocol

1. Day 1 - Aggregate protein following the normal procedure. This will yield protein at a final concentration of 4 µM. Set aside aliquots for 200 µL total that are aggregated simultaneously with those aggregated in the plate reader, but do not have ThioflavinS with them.
    - Note the presence of ThioflavinS at 100 µM has been shown to increase aggregation. We only use 20 µM ThioflavinS, but it is still best not to use the tau that has been used in the ThioflavinS aggregation assay.
2. Aggregate 5-6 hours at room temperature.
3. Add an equivalent volume of Ethanol to the unaggregated protein that has been kept on ice to the volume of Arachidonic Acid that was added to the aggregated protein.
4. Aliquot 20 µL of both the unaggregated and aggregated protein before spinning and mix 1:1 in 2X Laemmli Buffer. This will be total protein.
5. Spin all protein at 166,000 x g (30 psi) for 30 minutes.

6. Save supernatant (soluble protein). Bring final volume to 360 µL with 2X Laemmli Buffer and boil for 10 minutes. Store at -20°C for WB analysis.
7. Re-suspend pellet (Insoluble protein) in 100 µL of Sarkosyl Buffer.
    - Pellet is not visible so mark the "up" side of the tube.
    - This suspension should then be transferred to a 0.6 mL Eppendorf tube.
8. Vortex samples at room temperature for 30 minutes (tape to vortex).
9. Incubate overnight at 4°C with rocking.
10. Day 2 - Centrifuge the samples (Sarkosyl Soluble) for 1 hour at 166,000 x g.
11. Supernatant is soluble tau. Bring final volume to 360 µL with 2X Laemmli and boil for 10 minutes.
12. Resuspend pellet (Sarkosyl Insoluble) in 360 µL 2X Laemmli Buffer and boil for 10 minutes.
13. Analyze by Western blot.