Aggregation of Tau

References

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Solutions

1. Arachidonic acid solution: 4 mM Arachidonic Acid prepared in ice cold 200 proof ethanol
2. Aggregation buffer: 10 mM HEPES, 100 mM NaCl, 5 mM DTT

Abbreviations

1. AA: Arachidonic Acid
2. NaCl: Sodium Chloride
3. DTT: Dithiothreitol
4. dH_2O: Deionized water
5. EtOH: Ethanol

Protocol

1. Make FRESH arachidonic acid solution (Cat# 90010.1 from Cayman Chemical – stored at -20°C once opened).
    a. Wash AA designated pipet (which is labeled as such) with 10 rinses of ice cold 200 proof ethanol.
    b. Put 811 µL of ethanol in autoclaved Eppendorf on ice.
    c. Add 10 µL arachidonic acid (stored at -20°C) to ethanol.
    d. Final concentration = 4 mM.
    e. Rinse off pipet with 10 more rinses of ethanol.
2. Combine aggregation buffer with tau protein to make 500 µL:
    a. HEPES pH 7.4 to final concentration of 10 mM (20 µL 250 mM stock).
    b. NaCl to final concentration of 100 mM (20 µL 2.5 M NaCl).
    c. DTT (from 4°C) to final concentration of 5 mM (10 µL of 250 mM stock DTT).
    d. Tau to final concentration of 4 µm.
    e. dH_2O up to 500 µL.

3. Do not mix or pipet up and down or excess bubbles will result which will make it difficult to assess whether aggregation has occurred (using the laser).
4. Tilt to mix.
5. Add protein mixture to wells.
    a. Add 239 µL of each protein to 2 wells (one will be used as unaggregated control and the other will be aggregated).
    b. Then add 9.38 µL of either AA or EtOH (EtOH is added to the unaggregated wells) to each well to induce.
6. Incubate for 24 hours (may vary) at room temperature.
7. Store at 4°C.
8. FOR CULTURE:
    a. Airfuge samples for 15 minutes at 100,000 x g.
    b. Remove supernatant. Resuspend pellet in aggregation buffer. Let buffer sit with pellet for 1 hour on ice before transferring to another tube.
9. FOR WESTERN BLOT:
    a. Dilute 20 µL of sample 1:1 with 2x Laemmli Buffer and boil for 10 minutes.
    b. Load 20 µL of each sample and run on SDS-PAGE gel.