Aggregation of Tau as evaluated by ThioflavinS Fluorescence

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. ThS: Thioflavin S
6. 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. At the microplate reader, add 1 µL of ThS 5 mM for a final concentration of 20 µM.
    c. Then add 9.38 µL of either AA or EtOH (EtOH is added to the unaggregated wells) to each well to induce.
6. Read results on microplate reader for 5-6 hours.
    a. Excitation for ThS should be set to 440 nm.
    b. Emission should be set to 520 nm.