Tau aggregation protocol (MOK)

Materials:

* 0.2 um syringe filters for sterilizing (MCE membranes)
* 1.7 mL microcentrifuge tubes
* 384 well low volume black plate (Corning)
* SpectraMax M2/M5 plate reader
* D-PBS pH7.4, no Calcium or Magnesium (Life technologies cat# 14190)
* Magnesium chloride hexahydrate
* Thioflavin T (Sigma)
* Heparin, MW 6000-25000 (Santa Cruz, sc 203075)
* DTT
* Tau and chaperone protein buffer exchanged into the assay buffer

Preparation of working solutions:

(**Filter sterilize all solutions with a 0.2 um filter**)

* **1M MgCl2**: Dissolve Magnesium chloride hexahydrate in ddH2O. Store at room temp.
* **Assay buffer**: Add MgCl solution to D-PBS at a final concentration of 2 mM.
* **Thioflavin T (ThT) stock solution (0.5 mg/ml):** Dissolve Thioflavin T in assay buffer. Incubate at 37C for 20 min in dark of until ThT goes into solution. Dispense into single use aliquots and store at -20C
* **Heparin stock solution (0.22 mg/ml \* See notes ):** Measure out approx. 2 mg of heparin in an eppendorf. Dissolve in 1 ml assay buffer. Dilute the stock to make a solution of 0.22 mg/ml in assay buffer. Filter sterilize immediately. Store at 4C for up to 1 day.
* **100 mM DTT solution**: Dissolve DTT at 100 mM in assay buffer.

Assay conditions:

**General overview**: Thioflavin T is added to a Tau solution in DTT followed by heparin (inducer) or assay buffer (control). If chaperones are to be incubated with the tau solution, a 30 minute preincubation at 37C is carried out prior to addition of ThT and heparin.

The total volume per tube is 330 uL. All concentrations are listed as final concentration in assay.

-component 1: 10 uM Tau, 1mM DTT in assay buffer X uL

-component 2: 1mM DTT 3 uL

-component 3 (optional): chaperones X uL

-add assay buffer to total volume 297 uL

-component 4: 44 ug/ml heparin or assay buffer 33 uL

Total volume 330 uL

1. Mix Tau, DTT, chaperones (optional), and bring volume up to 297 uL. If doing multiple tubes , the entire solution+ inducer can be made in a falcon tube then quickly aliquoted into microcentrifuge tubes. If chaperones are added, incubate mixture for 30 min at 37C before adding heparin.
2. Add appropriate volume of heparin. If mixture needs to be aliquoted to tubes (330 uL aliquots), do so as quickly as possible.
3. Incubate tubes in 37C incubator on a shaker for 36h. Use highest rpm setting and variable shake mode.
4. The reaction can be checked for completion using a plate reader and ThT fluorescence. Make a ThT working solution of 10 uM. Quickly vortex fibril solution to evenly disperse. Take a 10 ul aliquot of the fibril solution using a p200 pipette tip (p10 tip opening too narrow) and mix with 10 uL of ThT working solution in a 384 well low volume plate. Incubate covered in the dark for 30 min then read with platereader. Take read with new fibril aliquot every hour until ThT fluorescence reading is stable (within 30 rfu)

Set up for the plate reader:

* + - 1. Fluorescence top read
      2. Plate=Corning 384 well low volume
      3. Read=well of interest
      4. Excitation 444 nm, Emission 485 nm, auto cutoff (480 nm)
      5. Medium PMT, 15 reads per well

1. Immediately after the aggregation protocol is complete, transfer the fibril solutions to **non-binding tubes.** Fibrils will gradually accumulate on normal eppendorf tube walls. Store fibrils at 4C

Other notes:

* Assay buffer should be uniform throughout assay. Buffering agent, salt, metals etc. can affect aggregation of tau or chaperone stability/function. If you use different buffers in your assay make sure you have tau only controls containing the same volume of buffer.
  + **All proteins must be dialyzed into the assay buffer. Even dilution of concentrated stock solutions of proteins can affect the aggregation kinetics if the buffers are not matched**
  + HEPES, and Tris buffers are not ideal because their pH changes greatly with temperature.
  + 140 mM salt is usually used. Higher salt conc. 300 mM completely blocks tau aggregation
  + The DTT in the buffer inhibits intramolecular disulfide bond formation in the tau molecule that impede intermolecular interactions.
  + Hsp70s and J-proteins may require Magnesium in assay for stability.
* The optimal ratio for heparin to tau to promote aggregation is 1:4. Since the heparin comes as a range of MW (6000-25000), this is difficult to calculate. For each batch of heparin, I test a range of concentrations and pick a concentration where the kinetics do not vary over at least a 4-fold range.
* ThT and heparin can not be premixed prior to addition to the assay. This greatly increasing the lag time for tau aggregation.
* Use regular eppendorf tubes. Tau will not aggregate in non-binding coated tubes. Tubes designed for 100K X g spins also have different kinetics from regular eppendorf tubes.