**Tau Disaggregase Assay Protocol**

This protocol is for assessing the ability of chaperones to disassemble preformed tau fibrils

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**Materials:**

* Preformed tau fibrils
* Aggregation assay buffer: DPBS + 2mM MgCl2
* ATP in assay buffer
* Chaperones dialyzed into assay buffer
* ThT stock in assay buffer (0.5 mg/mL, or 1568 uM)
* Corning 384 well low volume round bottom black plate (4511)
* SpectraMax M5 plate reader
* Ultracentrifuge tubes
* SDS-PAGE gel materials

**Disaggregation:**

1. Combine each of the following elements in an ultracentrifuge tube for the given final concentrations in a total volume of 220uL:
   1. Tau fibrils: 1uM monomer
   2. ATP: 5mM
   3. Chaperones: varies by type
      1. Hsp104: 10uM
      2. Hsc70: 1uM
      3. J protein: 0.5uM
      4. NEF: 0.1uM
   4. Assay buffer to bring up final volume
2. Incubate at 37C for 6 hours.

**Assessing disaggregation by ThT:**

1. Dilute ThT stock to 66.7uM by 1:23.5 dilution in assay buffer.
2. Combine 17uL of disaggregase reaction with 3uL of diluted ThT stock in a 384 well low volume plate. Final ThT concentration = 10uM.
3. Incubate covered in the dark for 30 min.
4. Read fluorescence with 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

**Assessing disaggregation by sedimentation:**

1. After removing samples for ThT analysis, ultracentrifuge the remaining volume at 100,000g for 1 hour at 4C.
2. Remove 150uL of supernatant and transfer to a new microcentrifuge tube. Combine 16uL of supernatant with 4uL of 5X SDS sample buffer.
3. Remove the remaining ~50uL of supernatant and discard.
4. Resuspend pellet with 220uL of 1X SDS sample buffer, then boil for 5 minutes.
5. Run 15uL each of the supernatant and pellet on a gel, and assess relative levels of tau in supernatant and pellet by gel quantification.