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Tau Thioflavin T Assay

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External link: https://www.stressmarq.com/support/technical-support/protocols/tau-protocol/

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Protocol status: Working
We use this protocol and it's working

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Materials

MATERIALS

- Thioflavin T **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T3516
- Active Human Recombinant Tau441 (2N4R) P301S mutant Protein Monomer **StressMarq Biosciences Catalog #**SPR-327
- Active Human Recombinant Tau (K18) P301L mutant Protein Monomer **StressMarq Biosciences Catalog #**SPR-328
- Active Human Recombinant Tau441 (2N4R) P301S mutant Protein Pre-formed Fibrils **StressMarq Biosciences Catalog #**SPR-329
- Active Human Recombinant Tau (K18) P301L mutant Protein Pre-formed Fibrils **StressMarq Biosciences Catalog #**SPR-330
- Microplate 96 well PS F-bottom (chimney well) black non-binding **greiner bio-one Catalog #**655900

1 A 1mM stock solution of Thioflavin T was prepared in dH2O (prepared fresh and filtered through a 0.2 µm syringe filter).

2 The thioflavin T was diluted in PBS pH 7.4 so that the final Thioflavin T concentration in each well was 25 µM (volume per well = 100 µL).

3 10 uM Heparin was added to each well.

4 Tau aliquots were thawed at room temperature just before use.

5 Either fibril or monomer (or both) was added to the appropriate wells. The well contents were pipetted up and down to mix.

6 The plate was sealed and placed in a shaking incubator (800 rpm) at 37 °C

7 Fluorescence was measured on a Molecular Devices Gemini XPS Microplate Reader using Softmax Pro software version 6.5.1.

XPS Microplate Reader Settings:

Temperature: 37°C
Read Type: Well Scan
Wavelength: Excitation at 450 nm and Emission at 485 nm
PMT Gain: Automatic
Flashes per read: 6
Shake: 20 seconds before read

8 The plate was re-sealed and placed into the shaking incubator at 37°C.

9 Readings were taking at regular intervals from 1 hour to 72 hours.