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Tau Thioflavin T Assay

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MATERIALS

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Thioflavin T **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**

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.wxgffjw

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

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protocols.io
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MANUSCRIPT CITATION:

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

Created: Jan 09, 2019

Last Modified: Jan 09, 2019

Keywords: Tau, Thioflavin T,
Fibrils, PFFs

- 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.