Tau aggregation monitored by thioflavin-T (ThT) fluorescence in a plate reader

Patricia Yuste-Checa[1], F Ulrich Hartl[1]

[1]Department of Cellular Biochemistry, Max Planck Institute of Biochemistry

Patricia Yuste-Checa

FEB 01, 2024

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.dm6gp3nw8vzp/v1

Protocol Citation: Patricia Yuste-Checa, F Ulrich Hartl 2024. Tau aggregation monitored by thioflavin-T (ThT) fluorescence in a plate reader. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp3nw8vzp/v1

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

Created: Jan 25, 2024

ABSTRACT

This protocol details how to efficiently monitor Tau aggregation by thioflavin T fluorescence in a plate reader.

ATTACHMENTS

Tau agg monitored by ThT in a plate reader.docx

MATERIALS

Buffers:

- Heparin: 50 undetermined in water, Heparin sodium salt from porcine intestinal mucosa (Merck, H3393). Can be stored at 4 °C. Prepare a fresh working dilution at 4.5 undetermined in water ([M] 250 micromolar (µM)).
- [M] 0.2 Molarity (M) MgCl2
- [M] 1 millimolar (mM) Thioflavin T (ThT). Can be stored at -20 °C.

Heparin sodium salt from porcine intestinal mucosa **Merck MilliporeSigma (Sigma-Aldrich) Catalog #H3393**

96 well half-area plate of black polystyrene with a clear bottom **Corning Catalog #3881**

Last Modified: Feb 01, 2024

PROTOCOL integer ID: 94537

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

Tau aggregation monitored by thioflavin-T (ThT) fluorescence in a plate re…

1 Mix reagents to a final concentration of [M] 10 micromolar (µM) Tau (TauRD), [M] 2.5 micromolar (µM) Heparin, [M] 2 millimolar (mM) MgCl2, [M] 10 micromolar (µM) ThT in 1x PBS pH 7.2. Prepare a mix for 4.5 reactions per condition (per condition, four technical replicates in each plate). Final volume is 80 µL per well.

> **Note**
>
> Molecular or chemical chaperones can be included in the reaction in order to study their effect on Tau aggregation.

2 Dispense 80 µL of the mix into a well of a 96 well half-area plate of black polystyrene with a clear bottom (Corning 3881).

3 If possible, the outer wells should not be used and instead filled with water.

4 Seal the plate with parafilm to avoid evaporation.

5 Set the following parameters in a SPARK multimode microplate reader (TECAN) and start the reaction:

- Fluorescence measurement: ThT signal, excitation 440 nm, emission 480 nm, (use gain regulation) measured every 2 minutes.
- Temperature 37 °C
- Constant shaking (50 seconds linear shaking: amplitude 4.5 mm, frequency 420 rpm - 50 seconds orbital shaking: amplitude 1.5 mm, frequency 360 rpm).

Note

- SPARK multimode microplate reader (TECAN) is highly recommended due to its high sensitivity and the gain regulation mode that increases the fluorescence detection window. When using other plate readers, the sample ThT signal easily gets saturated even when reducing initial gain to the minimum gain capacity.
- Cysteine-free TauRD (Tau residues 244-371, C291A/P301L/C322A/V337M), rapidly aggregates under these conditions, reaching the ThT plateau after 2 h aggregation.