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Thioflavin T Assay

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details Thioflavin T assay.

Attachments



[932-2408.docx](#)

17KB

Guidelines

Adapted from Alex Crowe, Jing Guo, Dustin Covell 032012 protocol, Mian Horvath Updates

Materials

Consumables:

- 1 mM Thioflavin T in MilliQ
- PBS
- Equipment needed: Spectrophotometer
- Black 96 well plate

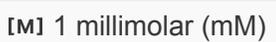
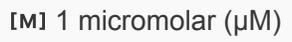
Thioflavin T Assay

1h

- 1 Resuspend fibril reaction. Fibrils will settle over time. Dilute  1.5 μL of  5 mg/mL fibrillization reaction 1:50 with PBS (total  75 μL). 

Note

Ideally, the same dilution of monomer, PBS alone and a previous batch of PFFs should be run in parallel. Mouse PFFs have low ThT fluorescence. In this case  5 μL PFFs can be diluted 1:10.

- 2 Assay each fibrillization in triplicate on the 96 well black assay plate. Dispense  20 μL of diluted α -synuclein fibrils per well. 
- 3 Dilute  1 millimolar (mM) Thioflavin T stock into PBS 1:1000 to obtain the required volume of Thioflavine T solution at a concentration of  1 micromolar (μM) . 
- 4 Dispense  180 μL of  1 micromolar (μM) Thioflavain T per well. 
- 5 Maintain plate at  Room temperature for  01:00:00 in the dark. 
- 6 Read plate on a Spectrophotometer excitation 450 nm, emission 510 nm, cutoff 475 nm.