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Thioflavin T Assay

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We use this protocol and it's working

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Abstract

This protocol details Thioflavin T assay.

Attachments

DOCX

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 [M] 1 millimolar (mM) Thioflavin T stock into PBS 1:1000 to obtain the required volume of Thioflavine T solution at a concentration of [M] 1 micromolar (µM) .

4 Dispense 180 µL of [M] 1 micromolar (µM) Thioflvain T per well.

5 Maintain plate at Room temperature for 01:00:00 in the dark. 1h

6 Read plate on a Spectrophotometer excitation 450 nm, emission 510 nm, cutoff 475 nm.