StressMarq Biosciences Inc.
PO Box 55036 Cadboro Bay | Victoria BC | V8N 4G0 CANADA
Tel: 250.294.9065 | Fax: 250.294.9025 |
info@stressmarq.com
www.stressmarq.com

Alpha Synuclein Protocols – Thioflavin T Assay

Protocol

Thioflavin T is a fluorescent dye that binds to beta sheet-rich structures, such as those in alpha synuclein fibrils. Upon binding, the emission spectrum of the dye experiences a red-shift and increased fluorescence intensity. The following protocol was used to generate the Thioflavin T assays using alpha synuclein preformed fibrils and monomers.

1. Prepare 1mM stock solution of Thioflavin T in dH2O (prepared fresh and filtered through a 0.2µm syringe filter).
2. Dilute Thioflavin T in PBS pH 7.4 so that the final concentration of ThioFlavin T in each well is 25 µM (volume per well = 100 µL). Plate: Lumox 96 multiwell plate (Sarstedt Catalog # 94.6000.024).
3. Thaw alpha synuclein aliquots at room temperature just before use.
4. Add either 10 nM preformed fibrils or 100 µM monomer (or both) to the appropriate wells. Pipet well contents up and down to mix. Concentrations are estimates and need to be optimized to give a good signal without being wasteful.
5. Seal plate and place in shaking incubator (600 rpm) at 37°C.
6. Measure fluorescence 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

7. Re-seal the plate and place into the shaking incubator at 37°C.
8. Take readings at regular intervals from 1 to 72 hours.