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

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

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Abstract

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

Attachments

DOCX

α-Synuclein agg moni...

96KB

Materials

Buffers:

- **α-Synuclein fibril buffer:**

	A	B
	NaN3	0.05%
	ThT	10 µM
	KCl	150 mM
	Tris-HCl pH 7.6	50 mM

- [M] 1 millimolar (mM) Thioflavin T (ThT). Can be stored at -20 °C .
- 6.5% NaN_3

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

Troubleshooting

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

14m

1 Mix reagents to a final concentration of [M] 200 micromolar (µM) α-Synuclein, 0.05 % NaN_3, [M] 10 micromolar (µM) ThT in α-Synuclein fibril buffer. 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 α-Synuclein 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.

3 If possible, the outer wells should not be used and should be filled them 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: 14m

- Fluorescence measurement: ThT signal, excitation 440 nm, emission 480 nm, (use gain regulation) measured every 00:10:00 .
- Temperature 37 °C
- Constant shaking (00:02:00 linear shaking: amplitude 1.5 mm, frequency 1080 rpm - 00:02:00 orbital shaking: amplitude 1 mm, frequency 510 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.
- Under these conditions α-Synuclein (A53T) slowly aggregates, reaching the ThT plateau after approximately 50 h aggregation.