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

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Aggregation of human recombinant alpha-synuclein

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ABSTRACT

This protocol describes a method of generating aggregation of human recombinant alpha-synuclein.

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1 Humanrecombinant α-Syn Monomeric WTα-Syn is purified from Escherichia coli as previously described in

CITATION

Hoyer W, Antony T, Cherny D, Heim G, Jovin TM, Subramaniam V (2002). Dependence of alpha-synuclein aggregate morphology on solution conditions..
LINK
https://doi.org/

[Hoyer et al., 2002].

2 Aggregation reactions are carried out using a solution of α-Syn [M] 70 micromolar (µM) in [M] 25 millimolar (mM) Tris buffervsupplemented with [M] 100 millimolar (mM) NaCl, pH 7.4 (in the presence of [M] 0.01 % volume % NaN3 to prevent bacterial growth).

3 The buffer is freshly prepared before each experiment and passed through a 0.02 µm syringe filter (Anotop, Whatman) to remove insoluble contaminants.

4 Prior to incubation, the reaction mixture is ultra-centrifuged at 90k r.p.m. for 01:00:00 at 4 °C to remove potential seeds. 1h

5 The supernatant is collected and separated in two fractions: one kept at 4 °C at all times until use(monomers), and a second incubated in the dark at 37°C 37 °C and 200 rpm, during 07:00:00 - 08:00:00 hours to avoid fibril formation (monomers+oligomers). 15h

6 α-Syn is always kept in LoBind microcentrifuge tubes (Eppendorf, Hamburg, Germany) to limit surface adsorption.