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Aggregation of human recombinant alpha-synuclein

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Protocol status: Working

We use this protocol and it's working

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Abstract

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



- 1 Humanrecombinant α -Syn Monomeric WT α -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.

<https://doi.org/>

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[Hoyer et al., 2002].

- 2 Aggregation reactions are carried out using a solution of α -Syn [M] 70 micromolar (μ M) 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 $\rightarrow$ 0.02 μ m 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 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.



Citations

Step 1

Hoyer W, Antony T, Cherny D, Heim G, Jovin TM, Subramaniam V. Dependence of alpha-synuclein aggregate morphology on solution conditions.

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