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# Recombinant a-synuclein pre-formed fibril generation

In 1 collection

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1 Works for me

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[dx.doi.org/10.17504/protocols.io.bu53ny8n](https://dx.doi.org/10.17504/protocols.io.bu53ny8n)

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## ABSTRACT

This protocol details the generation of a-synuclein pre-formed fibrils.

## ATTACHMENTS

[dh34b1qa7.pdf](#)

## DOI

[dx.doi.org/10.17504/protocols.io.bu53ny8n](https://dx.doi.org/10.17504/protocols.io.bu53ny8n)

## PROTOCOL CITATION

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## COLLECTIONS ⓘ

**Expression and purification of untagged asynuclein and recombinant a-synuclein pre-formed fibril generation**

## KEYWORDS

Recombinant a-synuclein pre-formed fibrils, Lyophilized monomeric synuclein

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## OWNERSHIP HISTORY

May 19, 2021 Urmilas

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50075


## PARENT PROTOCOLS

Part of collection

Expression and purification of untagged asynuclein and recombinant a-synuclein pre-formed fibril generation
















## MATERIALS TEXT

### Materials:

- Lyophilized monomeric synuclein  
Alternatively: aliquots of untagged alpha-synuclein in PBS, [M]0.5 mg/ml or [M]5 mg/ml
- Sterile dPBS
-  1.5 mL Protein LoBind tubes (Eppendorf)
- Parafilm

### Recombinant a-synuclein pre-formed fibril generation

1w 0d 0h 30m

- 1 Reconstitute  1 mg of lyophilized monomeric synuclein with  100 µl cold sterile DPBS  On ice (do not pipet, close tube immediately).
- 2 Transfer tubes on wet ice to  4 °C cold room and rotate on tube rotator for  00:10:00 . 10m
- 3  10m  
Centrifuge for  00:10:00 at  15000 x g at  4 °C .
- 4 Transfer supernatant to clean Protein-LoBind tube.
- 5 Prepare  5 µl aliquots of serial dilutions (1:10, 1:25, 1:50, 1:100) and measure concentration with Nanodrop (A280 - MW=14,5 kDa; Extinction coefficient  $\epsilon$  for human  $\alpha$ -syn = 5,960 M<sup>-1</sup>cm<sup>-1</sup>).
- 6 Dilute down to final concentration of [M]5 mg/ml , use  100 µl (  100 µl -  500 µl ) aliquots for reproducible results.
- 7 Take  1 µl -  2 µl aliquot of monomer and dilute to [M]1 ul/ml for electron microscopy, flash-freeze in dry ice – ethanol slurry.
- 8 Seal tubes with parafilm if no tube lock.

9



1w

Place the tube in an orbital thermomixer with a heated lid for 🕒 **168:00:00** at 🌡 **37 °C** , shaking at 🌀 **1000 rpm** .

**NOTE:** At the end of the 7 days, the contents of the tube should appear turbid. The thermomixer must have a lid to prevent condensation formation on the tube lids.

10



10m

Gently flick the tube to resuspend a-syn fibrils.

a. Fibrils for quality control steps can be stored at 🌡 **Room temperature** 🕒 **Overnight** .

11 Prepare aliquots for QC assays:

11.1 a. 📏 **5 µl** – thioflavin T binding assay.

11.2 b. 📏 **2 µl** for TEM.

12 For long-term storage (12-18 months), flash-freeze fibrils in liquid nitrogen and store at 🌡 **-80 °C** .