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Preparation of α-synuclein fibrils amplified from clinical material V.1

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ABSTRACT

This protocol describes the preparation of alpha-synuclein fibrils from clinical tissue with a quality control.
Optional conjugation step with phRodo STP ester dye is indicated

PROTOCOL CITATION

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MATERIALS TEXT

Protein LoBind Tubes, 1.5 Eppendorf tube mL **Eppendorf Catalog #0030108116** Step 3

PBS **Thermo Fisher Scientific Catalog #28374**

pHrodo™ iFL Red STP Ester (amine-reactive) **Thermo Fisher Catalog #P36011**

DMSO **Sigma Aldrich Catalog #472301**

Sodium bicarbonate **Sigma Aldrich Catalog #S6014**

1 Thaw down an aliquot of alpha-synuclein monomer stock (track down a batch number with identified EU number, concentration, A260/280 ratio) on ice

2 Spin down an aliquot of alpha-synuclein monomer stock solution (**20000 x g** , **00:10:00** **4 °C**) 10m and measure the concentration using nanodrop

Add **3 µL** of 10x diluted aliquot in PBS onto nanodrop pedestal;
Parameters: other proteins; coefficient extinction: 5.98; MW: 14.4 kDA
Perform two measurements and confirm <10% standard error between two measurements
If necessary, prepare 20X and 30X dilutions to confirm findings.

NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer
UV-Vis Spectrophotometer
Thermo Scientific **ND-ONE-W**

3 Calculate the reaction mix: **5 mg/mL** concentration of alpha-synuclein in **200 µL** in PBS (use safe-lock eppendorf tubes Protein LoBind Tubes, 1.5 mL **Eppendorf Catalog #0030108116** or other Thermo Scientific™ Low Protein Binding Collection Tubes (1.5 mL) **Contributed by users Catalog #PI90411**
)

3.1 Prepare a reaction mix containing 10% of clinical tissue and **5 mg/mL** of alpha-synuclein in PBS

4 5d

Incubate the reaction at **1000 rpm, 37°C** for **120:00:00** using program settings: 1 min ON; 1 min OFF

Eppendorf Thermomixer C Model 5382
Thermomixer C
Eppendorf **5382000023**

ThermoTop®
Smart block
Eppendorf **5308000003**

5 10m

Spin down the insoluble fraction at **15000 rpm, 10°C, 00:10:00** ; take **100 µL** of supernatant and add **1 mL** of fresh PBS to the pellet

Example of reaction mix after 5 days of incubation

6 10m

Gently resuspend and spin down the fibrils at **15000 rpm, 10°C, 00:10:00** ; take **100 µL** of supernatant and add **1 mL** of fresh PBS to the pellet (repeat 3 times)

7

Step 7 includes a Step case.

Conjugation of full-length fibrils with pHrodo dye

step case

Conjugation of full-length fibrils with pHrodo dye

1. Dissolve 100 ug of dye in 50 ul of sterile DMSO (2 mg/mL)
2. Dilute fibrils to 1mg/mL concentration in PBS containing 0.1M bicarbonate (total volume: 0.95 mL)
3. Add 50 ug of dissolved dye to each reaction tube
4. Incubate overnight in eppendorf tube (foil-wrapped) with continuous shaking
5. In the morning, spin down the dye-fibril solution at 10,000 g for 10 min at 10C
6. Transfer the supernatant and dissolve the pellet with 1 mL of PBS (repeat 5 times)

8 Take out 900 ul of PBS leaving 100 ul of fibril pellet in the tubes. Transfer the fibrils into 0.6 mL PCR tubes (thick wall).

9 Sonicate the fibrils using water bath sonicator at 10C, 30% amplitude and for 1 hour (no OFF ON cycles). Check the level of water in the water-bath is in a line with the tube content.

10 Measure size of sonicated particles using DLS

Dynamic Light Scattering measurements

by arpine.sokratian

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