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α-synuclein PFF treatment

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We use this protocol and it's working

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Abstract

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1 Shake Alpha-Synuclein Monomers in PBS for 7 days at 1,000 RPM. Solution should turn turbid during this period.

2 Spin fibrils for 10 minutes at max speed in benchtop centrifuge at Room temperature.

3 Discard supernatant and resuspend fibrils in PBS at a concentration of 5 ug/ul.

4 Validate α-synuclein PFFs via:
- Electron microscopy
- Sedimentation assay
- Thioflavin-T assay (Cat. number T3516; Sigma–Aldrich)

5 Label α-synuclein PFFs with an Alexa Fluor 594 kit (Cat. number A10239; Thermo Fisher Scientific) following the manufacturer's instructions.

For microglial treatment:

6
- Dilute α-synuclein PFFs in PBS to 100 µg/mL.
- Sonicate the solution (10-second pulses at 30% amplitude, six times with 2-minute intervals) using a Q700-220 sonicator (Qsonica, Newtown, CT, USA).
- Dilute sonicated PFFs in microglial medium to a final concentration of 1 µg/µL.

7 Add α-synuclein PFFs to iPSC-derived microglia cultures and cotreat with CDDO-Me, etoposide, or MCHA as needed.

8 Fix cells with 4% PFA for 10 minutes after 30, 60, and 90 minutes of treatment.

9 Perform immunostaining as described in the paper.

10 Acquire images:
- For immunostained samples: use a Leica TCS SP8 confocal microscope with a 60×/1.4 numerical aperture oil immersion objective.
- For live-cell imaging: use an Evident APX100-HCU box-type microscope with a 20×/0.8 numerical aperture objective. Capture images every 20 minutes for 3 hours.

11 For quantitative analysis:
- Select three random fields containing 5–15 cells per field.
- Image and analyze these fields across 3 independent experiments.

12 Perform particle analysis using Speckle Counting and Object Tracking pipelines in CellProfiler Image Analysis Software (version 4.2.6, RRID: SCR_007358) according to recommended guidelines.

13 Detect cell bodies using CD68 staining or brightfield imaging.