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Preparation of fibrils for intracerebral injection

In 1 collection

The Michael J Fox Foundation Pff Standardization Consortium[1]

[1]MJFF 2017 Committee

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dx.doi.org/10.17504/protocols.io.bws4pegw

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ABSTRACT

This is a consensus protocol developed through discussions with Laura Volpicelli-Daley, Caryl Sortwell, Kelvin Luk, Lindsey Gottler, and Virginia Lee. This protocol is intended for research purposes only, using specially-formulated monomeric alpha-synuclein protein available for purchase at Proteos, Inc as the result of efforts by The Michael J. Fox Foundation (MJFF). Each batch of the “Alpha-Synuclein Monomer Protein for Making Pre- Formed Fibrils” has undergone internal purification and quality control at Proteos in addition to external validation to confirm successful generation of pathogenic aSyn PFFs. See Reference section for methods and results from application of alpha-synuclein pre-formed fibrils (aSyn PFFs) in primary neuron cultures in vitro or in mice in vivo. This protocol is referenced in the Polinski et al 2018 paper entitled "Best Practices for Generating and Using Alpha-Synuclein Pre-Formed Fibrils to Model Parkinson's Disease in Rodents" (doi: 10.3233/JPD-171248).

ATTACHMENTS

dz3jbh9f7.pdf

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PROTOCOL CITATION

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COLLECTIONS

Protocol for Generation of Pre-Formed Fibrils from Alpha-Synuclein Monomer

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Protocol for Generation of Pre-Formed Fibrils from Alpha-Synuclein Monomer

MATERIALS TEXT

NOTE: Prior to in vivo or in vitro use, it is highly recommended that sonication parameters be established to result in fibrils of <= 50nm in length (as longer fibrils result in limited or lack of toxicity). Fibril length can be verified using electron microscopy or dynamic light scattering. Once appropriate sonication parameters are identified to result in fibrils of <= 50nm in length, these parameters should be strictly followed. A change in PFF sample volume or concentration may require modified sonication parameters.

Reagents:

- We have successfully elicited synuclein pathology following injection withfibrils in C57Bl/6, CD1, C57Bl6/Sv129, and C57B/C3H mice.
- Sterile dPBS
- [M]**5 mg/ml** aSyn PFFs (as prepared in Step 1). Thaw at room temperature immediately before use.

Equipment:

- Fume hood (BSL2)
- Sonicator with 1/8" tip (Qsonica XL-2000)
- Stereotaxic surgery setup

Preparation of fibrils for intracerebral injection. Perform ~1-2 hours prior to surgery. This takes about 30 min.
1m

1 Perform all sonication steps in a fume hood or biosafety cabinet.

Ensure that hood is externally ducted and does not re-circulate exhaust into the laboratory space.

2 Thaw sufficient aliquots of [M]**5 mg/ml** PFFs at **Room temperature** immediately before use.

It is recommended to measure protein concentration again (See Step 1, Protocol Step 3) as freeze-thaw may change protein concentration.

3 Dilute PFFs to required concentration by adding PFFs to a sterile microcentrifuge tube containing the appropriate volume of sterile dPBS.

Note that pffs are assembled in dPBS. For mouse injections, we typically use [M]**2 mg/ml** - [M]**2.5 mg/ml** PFFs.

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4 Using a probe sonicator, sonicate at power level 2 for a total of 60 pulses (~0.5 seconds each). Pause briefly between every 10-12 pulses to prevent solution from heating up excessively and to avoid frothing.

NOTE: We have also tested this protocol using select high-energy bath sonicators such as the Covaris and Bioruptor systems. Results so far indicate that they are also suitable for preparation of PFFs prior to addition/injection. These systems can sonicate closed tubes and are preferable where aerosol generation is a concern. However, be cautious when using bath sonication in place of probe sonication and be sure to verify fibrils are ≤ 50nm in length for proper toxicity.

5 Close cap and tap side of tube so that any liquid on the side of the tube is now at the bottom.

It should appear clear and colorless, although small fragments that scatter light may still be visible.

6 Allow sonicated PFF solution to settle for **00:01:00** . 1m

PFF suspension is now ready for stereotaxic injection.

7 Gently flick tube to mix contents prior to use and pipette up and down between surgical injections. It is recommended to use an aliquot for up to 4 hours during a surgical session. If an 8 hour surgical session is planned, use one aliquot in the morning and a new aliquot in the afternoon.

NOTE: If aSyn monomers are being used as the control, be sure endotoxin units (EUs) are near or below 0.5 EU/mL. The Pierce High Capacity Endotoxin Removal Kit is a reliable method for removing endotoxins. Please note you may lose a good portion of your sample in the process and should re-measure protein levels after endotoxin cleanup.

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