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

The Michael J Fox Foundation Pff Standardization Consortium[1]

[1]MJFF 2017 Committee

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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

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OPTIMAL CONDITIONS FOR PFF GENERATION

Ionic strength and pH are very important to monitor when generating aSyn PFFs. These two parameters may affect PFF formation and should be analyzed prior to PFF induction. The pH should be ~7.0 and the salt concentration should be approximately 100mM NaCl. Proper sonication is extremely important. The parameters of this protocol are designed for use with the Proteos protein and sonication with the instruments listed. For optimal seeding, fibrils should be 50nm or smaller in length after sonication. For information on other sonication parameters, see Reference 8.

STORAGE OF PFFS

PFFs should be aliquoted into single-use tubes. Aliquots of aSyn PFFs may be stored at room temp or -80°C. PFFs should NOT be stored at 4°C. If aliquots are kept at room temp, ensure that sterile components were used to assemble reactions to prevent microbial contamination. Sodium azide can also be added as a preservative if compatible with downstream application but may result in cell toxicity. If aliquots are stored at -80°C, precautions must be made to avoid unnecessary freezethaw—keep samples in a box towards the back of the freezer and minimize the amount of time the box is open at room temp. Freeze-thaw cycles will degrade PFFs by dissociating the fibrils and/or causing non-specific aggregates. Properly-stored PFFs have been shown to lose pathogenicity after 1-1.5 years at -80°C. For in vivo studies, it is highly recommended to use freshly-made PFFs.

RECOMMENDED QUALITY CONTROL MEASURES FOR PFFS

Various biochemical and biophysical quality control experiments are recommended to ensure proper aSyn PFF formation. The necessity for each experiment is dictated by experience with the PFF generation protocol and the designated use of the PFFs.

Purpose	Circumstances for Performing	Examples of Experiments	Anticipated Results
Verify Fibril Size	When using a protocol for generating PFFs for the first time. Prior to long-term *in vivo* studies.	Electron Microscopy	Majority of fragments are ≤ 50nm
		Dynamic Light Scattering	Majority of fragments are ≤ 50nm
Verify Seeding Capacity	When using a protocol for generating PFFs for the first time. Prior to long-term *in vivo* studies.	*In vitro* Seeding in Mouse Primary Neuron Cultures	pS129 pathology develops in primary neurons following aSyn PFF, but not aSyn monomer, incubation
Verify Fibril Formation	With each new batch of PFFs or prior to using frozen aliquots	Thioflavin T Assay	Readings 20-100 fold higher with human aSyn PFFs vs monomer
		Sedimentation Assay	Equal amounts of protein in solute and pellet or more protein in pellet vs solute
		Visual Appearance	Solution should appear turbid

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FILES

Preparation of fibrils and Quality control
Version 1
by Sonya Dumanis

Preparation of fibrils for intracerebral injection
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