**Induction of aggregation in alpha-synuclein-expressing cells by treatment with preformed fibrils (PFFs)**

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**Abstract**

This protocol details how to efficiently produce alpha-synuclein aggregates in cells by templating the misfolding of intracellular alpha-synuclein through treatment with preformed fibrils of alpha-synuclein (PFFs).

Keywords: alpha-synuclein, preformed fibrils, PFF, Parkinson’s Disease

**Materials**

Cell culture

Cells expressing alpha-synuclein

Reagents

* Appropriate cell culture medium
* Lipofectamine 3000 (Thermo Fisher Scientific cat. no. L3000008)
* OptiMEM (Thermo Fisher Scientific cat. no. 31985062)
* Phosphate-buffered saline (PBS) (Thermo Fisher Scientific cat. no. 20012068)
* 5 mg/ml alpha synuclein preformed fibrils (PFFs) (see dx.doi.org/10.17504/protocols.io.btynnpve for purification protocol)

Equipment

* BioRuptor Plus sonicator (Diagenode cat. no. B01020001) (or equivalent)

**Induction of alpha synuclein aggregation**

1. Seed cells into plates such that they are ~25% confluent the following day.
	1. For our HEK cells, the correct seeding density is ~250 cells/mm2, which is equivalent to 100,000 cells in a 12-well dish.
2. On the following day, warm PBS and OptiMEM to 37C, thaw an aliquot of PFFs, and (if using) allow lipofectamine to reach room temperature.
	1. Note: Lipofectamine greatly increases the ability of PFFs to nucleate intracellular alpha synuclein aggregation, perhaps by altering the endocytic route that PFFs use to enter the cell. It is therefore not recommended to use lipofectamine if the route of PFF entry is a concern in your experiment. Lipofectamine is additionally excessively toxic and should therefore be avoided in some cell types.
3. Dilute the thawed PFFs 1:20 into an eppendorf containing PBS.
	1. Note: the final volume must be between 100 and 300 ul for proper sonication in the Bioruptor Plus.
4. Sonicate the PFFs in the Bioruptor Plus on high for 25 cycles of 5 seconds on and 5 seconds off at 4C.
	1. Note: Sonication breaks up the PFFs into smaller, more nucleation-competent fibrils.
5. Make a master mix of PFF and PBS solutions
	1. 20 ul of sonicated & diluted PFFs should be added per mm2 of plate area (5 ug alpha-synuclein/mm2)
		1. For a 12-well plate, this would be 40 ul of sonicated & diluted PFFs.
	2. Master mixes should contain a ratio of 50 ul OptiMEM : 20 ul sonicated & diluted PFFs (or PBS as a control) : 3 ul of lipofectamine 3000
		1. For a 12-well plate, 100 OptiMEM : 40 ul sonicated & diluted PFFs/PBS : 6 ul lipofectamine 300 should be used.
	3. If using lipofectamine, first incubate the lipofectamine 3000 in OptiMEM for 5 minutes before adding PFFs or PBS. Gently vortex upon addition of lipofectamine 3000.
6. After addition of PFFs/PBS, gently vortex master mix solutions and incubate for 10 minutes at room temperature.
7. Add master mix solutions dropwise to cells, gently vortexing before adding to each well.

**Relevant references**

Luk KC, Song C, O'Brien P, Stieber A, Branch JR, Brunden KR, Trojanowski JQ, Lee VM. Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc Natl Acad Sci U S A. 2009 Nov 24;106(47):20051-6. doi: 10.1073/pnas.0908005106. Epub 2009 Nov 5. PMID: 19892735; PMCID: PMC2785290.

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