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

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Cole S Sitron[1], Victoria A Trinkaus[1], F Ulrich Hartl[1]

[1]Department of Cellular Biochemistry, Max Planck Institute of Biochemistry

C Cole Sitron
Max Planck Institute of Biochemistry

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We use this protocol and it's working

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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).

Attachments

DOCX

957-2488.docx

16KB

Materials

Cell culture

Cells expressing alpha-synuclein

Reagents

- Appropriate cell culture medium
- Lipofectamine™ 3000 Transfection Reagent **Thermo Fisher Scientific Catalog #**L3000008
- Opti-MEM™ Reduced Serum Medium **Thermo Fisher Scientific Catalog #**31985062
- PBS, pH 7.2 **Thermo Fisher Scientific Catalog #**20012068
- [M] 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)

Equipment	
Bioruptor® Plus sonication device	NAME
Sonication device	TYPE
Bioruptor®	BRAND
B01020001	SKU
https://www.diagenode.com/en/p/bioruptor-plus-sonication-device	LINK

Induction of alpha synuclein aggregation

15m 10s

1 Seed cells into plates such that they are ~25% confluent the following day.

Note

For our HEK cells, the correct seeding density is ~250 cells/mm^2, which is equivalent to 100,000 cells in a 12-well dish.

2 On the following day, warm PBS and OptiMEM to 37 °C, thaw an aliquot of PFFs, and (if using) allow lipofectamine to reach Room temperature.

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.

Note

The final volume must be between 100 µL and 300 µL for proper sonication in the Bioruptor Plus.

4 Sonicate the PFFs in the Bioruptor Plus on high for 25 cycles of 00:00:05 on and 00:00:05 off at 4 °C.

10s

Note

Sonication breaks up the PFFs into smaller, more nucleation-competent fibrils.

5 Make a master mix of PFF and PBS solutions.

5.1 1 µL of sonicated & diluted PFFs should be added per 10 mm^2 of plate area (5 ug alpha-synuclein/10 mm^2).

Note

For a 12-well plate, this would be 40 µL of sonicated & diluted PFFs.

5.2 Master mixes should contain a ratio of 50 µL OptiMEM : 20 µL sonicated & diluted PFFs (or PBS as a control) : 3 µL of lipofectamine 3000.

Note

For a 12-well plate, 100 OptiMEM : 40 µL sonicated & diluted PFFs/PBS : 6 µL lipofectamine 300 should be used.

5.3 If using lipofectamine, first incubate the lipofectamine 3000 in OptiMEM for 00:05:00 before adding PFFs or PBS. Gently vortex upon addition of lipofectamine 3000.

5m

6 After addition of PFFs/PBS, gently vortex master mix solutions and incubate for 00:10:00 at Room temperature .

10m

7 Add master mix solutions dropwise to cells, gently vortexing before adding to each well.

Protocol references

1. 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.

2. Volpicelli-Daley LA, Luk KC, Lee VM. Addition of exogenous α-synuclein preformed fibrils to primary neuronal cultures to seed recruitment of endogenous α-synuclein to Lewy body and Lewy neurite-like aggregates. Nat Protoc. 2014 Sep;9(9):2135-46. doi: 10.1038/nprot.2014.143. Epub 2014 Aug 14. PMID: 25122523; PMCID: PMC4372899.