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Filter trap assay for the detection of alpha-synuclein aggregation

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes an assay that detects aggregated alpha-synuclein in cell lysates.

Materials

Cell culture

Cells with alpha-synuclein aggregates, induced as in [dx.doi.org/10.17504/protocols.io.eq2lyjbbplx9/v1], growing in 12-well plates.

Reagents

- TrypLE™ Express **Thermo Fisher Scientific Catalog #**12605010
- Pierce™ Rapid Gold BCA Protein Assay Kit **Thermo Fisher Catalog #**A53225

Buffers

- 10% FBS (Fetal Bovine Serum **Gibco - Thermo Fisher Scientific Catalog #**10270106) in 1X PBS (diluted from PBS (10X), pH 7.4 **Thermo Fisher Scientific Catalog #**70011051)
- 1X PBS
- Lysis Buffer:

	A	B
	Tris-HCl pH 7.5	50 mM
	NaCl	150 mM
	Triton-x100	1% (v/v)
	cOmplete protease inhibitor tablet	1 mini tablet per 15 ml buffer (Sigma-Aldrich cat. no. 4693159001)
	Phos-STOP phosphatase inhibitor tablet	1 tablet per 15 ml buffer (Sigma-Aldrich cat. no. 4906845001)
	Benzonase ((Max Planck Institute of Biochemistry Core Facility)	7.5 U/ml

- Wash Buffer:

	A	B
	Tris-HCl pH 7.5	50 mM
	NaCl	150 mM
	Triton-x100	1% (v/v)

Consumables

- 0.2 micrometer pore size cellulose acetate membrane (e.g. GE cat. no. 10404131)
- PARAFILM® M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P7793

Equipment

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

- Slot blot vacuum manifold (e.g. Hoefer PR648)
- Fume hood

Harvesting cells

1 Prepare lysis buffer and place On ice .

2 After 24:00:00 of exposure to PFFs/PBS, wash wells 1X with 1 mL warm PBS. 1d

3 Withdraw PBS and treat wells with 300 µL of TrypLE.

4 Quench dissociation reagent with 300 µL of 10% FBS and transfer the contents of the wells to 1.5 ml tubes.

5 Centrifuge 1500 x g for 00:03:00 . 3m

6 Withdraw the supernatant and wash cells with 1 mL PBS.

7 Centrifuge 1500 x g for 00:03:00 . 3m

8 Withdraw supernatant and resuspend in 100 µL of lysis buffer and incubate On ice for 00:05:00 . 5m

9 Sonicate the cells in the Bioruptor for 3 cycles of 00:00:30 on and 00:00:30 off at high power. 1m

10 Centrifuge the lysate at 500 x g for 00:05:00 and place the clarified lysate in a new 1.5 ml tube. 5m

Note

This step is crucial to ensure the removal of high-diameter material (e.g. unlysed cells) that may clog the filter paper and non-specifically trap proteins on it.

Note

If desired, this is a convenient stopping point, wherein you can snap-freeze your lysate in liquid nitrogen and proceed with the rest of the assay at a later time.

11 Determine the concentration of protein in each sample by Pierce Rapid Gold BCA Protein Assay.

Filter Trap

10m

12 Soak the cellulose acetate membrane in wash buffer for > 00:10:00 . 10m

13 For each well that you run, prepare 1020 µL of the sample containing 10.2 - 51 ug of lysate, with the remaining volume consisting of lysis buffer.

14 Assemble the slot blot vacuum manifold apparatus with the cellulose acetate membrane.

15 Wash each well with 300 µL wash buffer, inspecting each well to ensure that the buffer can drain through it.

Note

Avoid using any non-functioning wells.

16 Load 1000 µL (10 - 50 ug) of sample to each well.

17 Once the sample has drained through the wells, wash each well 3 times with 300 µL of wash buffer.

18 When all of the washes have drained through the wells, trim the membrane and proceed with standard immunodetection methods.