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Sedimentation Assay

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lyjb9mlx9/v1

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

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Abstract

This protocol details sedimentation assay for alpha-synuclein fibrils.

Attachments

PDF

932-2407.pdf

360KB

With Sucrose Cushion

1

Note

A sucrose cushion will minimize chances of the pellet being disturbed but may increase the chances of a-synuclein monomer ending up in the pellet fraction.

Dilute 4 µL of 5 mg/mL α-synuclein PFFs to 40 µL in PBS in ultracentrifuge tubes.

2 Add 40 µL 20% sucrose beneath PFFs.

3 Ultracentrifuge at 100000 x g (45000 rpm) for 00:30:00 at 22 °C . 30m

4 Remove 70 µL supernatant and add to new tube.

5 Add 60 µL 12.5% sucrose to the pellet and resuspend the pellet by pipetting.

6 Dilute samples in 5x sample buffer.

7 Boil samples at 95 °C for 00:05:00 . 5m

8 Run samples on a 15% polyacrylamide gel.

Note

20 µL or less of sample can be loaded. Loading less may make for a cleaner gel (no α-synuclein PFFs visible in the supernatant).

9 Stain with Coomassie blue.

Without Sucrose Cushion

35m

10 Dilute 4 µL of 5 mg/mL α-synuclein PFFs to 40 µL in PBS.

11 Ultracentrifuge at 100000 x g (45000 rpm) for 00:30:00 at 25 °C . 30m

12 Remove supernatant and dilute in 5x sample buffer.

13 Add 40 µL PBS to the pellet.

14 Resuspend pellet by pipetting up and down. Dilute in 5x sample buffer.

15 Boil samples at 95 °C for 00:05:00 . 5m

16 Run samples on a 15% polyacrylamide gel.

> Note
>
> 10 µL or less of sample can be loaded. Loading less may make for a cleaner gel (no α-synuclein PFFs visible in the supernatant).

17 Stain with Coomassie blue.