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Sedimentation Assay

 In 1 collection

DOI

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

Michael X. Henderson¹

¹Van Andel Institute



Michael Henderson

Van Andel Research Institute

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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



[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 α -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 $^{\circ}\text{C}$.

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 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$.

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 $^{\circ}\text{C}$ 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.