

JAN 24, 2024

# Sedimentation Assay



In 1 collection

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#### **ABSTRACT**

This protocol details sedimentation assay for alpha-synuclein fibrils.

#### **ATTACHMENTS**

932-2407.pdf





#### DOI:

dx.doi.org/10.17504/protocols.io. eq2lyjb9mlx9/v1

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

Created: Dec 20, 2023

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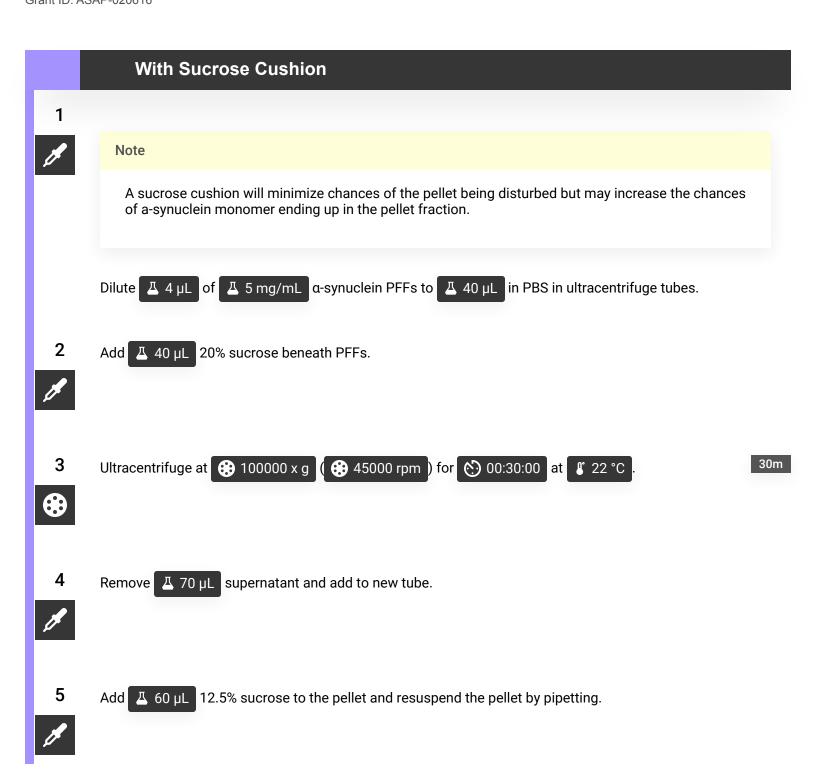


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PROTOCOL integer ID: 93623

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- **6** Dilute samples in 5x sample buffer.
- 7 Boil samples at \$\mathbb{8}^\* 95 \cdot \cdot \cdot \cdot 00:05:00

5m

**8** Run samples on a 15% polyacrylamide gel.



### Note

 $\bot$  20  $\mu$ L or less of sample can be loaded. Loading less may make for a cleaner gel (no  $\alpha$ -synuclein PFFs visible in the supernatant).

**9** Stain with Coomassie blue.

## **Without Sucrose Cushion**

35m

- Dilute  $\triangle$  4  $\mu$ L of  $\triangle$  5 mg/mL  $\alpha$ -synuclein PFFs to  $\triangle$  40  $\mu$ L 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.

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13 Add  $\perp$  40  $\mu$ L PBS to the pellet.



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



15 Boil samples at \$\ \ \ 95 \ \ \ for \ \ \ 00:05:00 \]



16 Run samples on a 15% polyacrylamide gel.



Note

I 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.

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