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Denaturation analysis of α-synuclein fibrils

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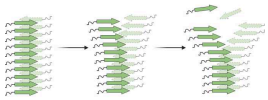

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details the specific parameters used to accurately measure the denaturation sensitivity of α-synuclein fibrils. Essential parts of this protocol describe the evaluation of sonicated fibrils to ensure the reproducibility of the results. Visualization of α-synuclein is recommended to be conducted using slot-blot analysis with aggregate-specific α-synuclein antibodies.

Protocol materials

- Guanidine Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G3272
- Sodium Dodecyl Sulfate (SDS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**436143
- N-Lauroylsarcosine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**61739
- Thermo Scientific™ Low Protein Binding Collection Tubes (1.5 mL) **Catalog #**PI90411
- Alexa-647-MJFR14-6-4-2 **Abcam Catalog ##**ab216309

Safety warnings

Hazard Identification and Risk of Exposure to the
Hazards:

Inhalation or spread through food or drink that contain fibrils aerosols or fibrils.
Protective gloves, safety glasses and lab coat must always be used when handling anything that possibly could contain α-synuclein fibrils. Food or drink is strictly prohibited in any environment where α-syn fibrils are used.

Routes of Transmission: *Prior to assigning containment requirements, it is imperative to understand the routes of transmission.*

Some issues to address:

1. What are the exposure routes/risks of most concern:

Inhalation or spread through food or drink that contain fibril aerosols or fibirls accordingly. Fibrils possibly might reach the brain regions through the olfactory epithelium; Risk of accidental needlestick/droplet splash while handling fibrils for *in vitro* or *in vivo* work.

1. *What are the consequences of exposure (potential illness, etc)*

Fibrils may be considered as infectious material. Minimum to no hazard is expected from α-syn protein. *There is no evidence that transmission of fibrils can lead to development Parkinson's disease.* However, taking into account prion-like properties of α-syn fibrils should therefore be handled cautiously and wisely. Strictly recommended using disposable materials and Personal Protective Equipment (PPE) such as gloves, face mask, etc.

PRECAUTIONS:

Laboratory work where high concentration of fibrils (more than 300 uM) is needed must comply with biosafety level 2 (BSL2) containment as described in the current edition of the CDC/NIH's Biosafety in the Microbiological and Biomedical Laboratories: *http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm*

Sharps safety precautions:

The use of sharps (glass pipettes, glass slides and cover slips, scalpels and lancets) should be eliminated, when possible. Appropriate precautions should be taken to avoid percutaneous injuries. These items should be disposed of immediately in a puncture-resistant sharps container. Bending, recapping or clipping of needles is prohibited. As described in CDC's sharps safety website: https://www.cdc.gov/sharpssafety/index.html

Procedural Methods and Materials:

- Laboratory work where high concentration of fibrils (more than 300 uM) is needed must comply with biosafety level 2 (BSL2) containment. This means all aerosol generating procedures must be

performed within the biosafety cabinet.

- *All the fibrils work involves using PPE, aerosol-tight centrifuges, water bath sonicator in a closed cabinet, homogenization of frozen brain samples using probe-tip sonicator under the hood (collection of protein fractions in BSL2 cabinets), chromatography equipment in a closed-door fridge, sealed plates, safe lock microfuge tubes (or tubes wrapped/sealed with parafilm), and use of filtered tips for pipettes. All personnel must strictly adhere to these procedures.*
- Use of proper PPE as stated in the section below. Use of available N95 respirators is voluntary (same for the use of available sleeve protectors). Follow safety precautions for sharps (for e.g., to avoid accidental needle sticks) while working with PFFs in the lab and for doing *in vivo* work.

Personal Protective Equipment (PPE): Appropriate PPE includes gloves, lab coat and safety glasses, face mask (voluntary N95 respirator use and sleeve protectors), face / bench top splash shield for specific procedures as stated above.

Methods to minimize personal exposure: Strictly adhere to sharps safety precautions using needles or any material that can potentially cause wounds. Use disposable supplies where possible. Use the minimal amounts of α-fibrils needed for an experiment. Keep fibrils in closed tubes. 10% of SDS solution in water must be used for decontaminating work areas. Do not use NaOH or Sodium Hypochlorite or ethanol. Do not leave samples containing fibrils unattended at the bench.

Methods to prevent the release of fibrils/protect workers from aerosols, splashes, splatters: protective gloves and clothing always be always be worn when handling frozen vials. High concentration of fibrils(>1mg/mL) always be handled under Biosafety cabinet and containment caps will be used while centrifugation. Centrifuge cups will be opened inside a biosafety cabinet. Face shield or benchtop splash shield will be used when working at the open bench.

Specimen transport and removal of material(s) from the laboratory: Transported in secondary container (plastic/Styrofoam) in a closed box. The closed box is carried in a bag.

Standard microbiological methods: hand washing after removal of gloves and before leaving the work area, no mouth pipetting, strictly no food or drink in refrigerators where material is stored, no eating in work area.

Cleaning & Disinfection: Work area must be cleaned with *10% SDS in water.* Wipes used must be immediately disposed into biohazard waste container. Any piece of equipment or supplies that possibly have been exposed to fibrils must be wiped with 10% of SDS.

Waste Generation and Disposal Methods: The solutions that contain α-syn fibrils must be decontaminated with 10% of SDS in water for 30 minutes and be thrown as a biohazard waste in a sealed container/bag (use a minimal volume of fibrils needed for an experiment, do not generate large volumes of fibril-containing liquids). Use small biohazard bags to collect tips and consumables of experiment performed, appropriately tie neck of bag in single knot and place in into secondary biohazard waste container.

Spill and Accident Response Procedure: *Describe all emergency procedures including spill clean-up. Describedisinfectants and environmental decontamination.* (ex., Outside of a BSC: If spill is a respiratory hazard, evacuate 30 minutes to allow aerosols to settle. Place absorbent towels over the spill, apply freshly prepared 10% SDS solution to entire area of spill starting on the outer edges and working inward, pick up sharp items with mechanical device (not hands), place all clean-up materials in a biohazard bag)

Preparation of α-synuclein sonicated fibrils

1 Follow the protocol to prepare the batch of sonicated fibrils. If the samples have been prepared earlier and kept at -80, thaw down in a water bath. Then, perform a quality control check to ensure the size distribution and concentration are corresponding to values outlined in the protocol attached.

Protocol

NAME

Preparation of mouse and human α-synuclein fibrils

CREATED BY

Arpine Sokratian

Preview

2 Evaluated sonicated fibrils should be diluted in PBS to concentration of 5 mg/mL.

Preparation of denaturating agents

3 Prepare stock solutions of
6M Guanidine HCL

Guanidine Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G3272

10% SDS -

Sodium Dodecyl Sulfate (SDS) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**436143

10% Sarkosyl -

N-Lauroylsarcosine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**61739

4 Dilute the chemical solutions:
6M, 2M, 1M, 0.5M, 0.25M, 0.125M for GuHCL
2%, 0.2%, 0.02%, 0.002%, 0.0002%, 0.00002%
8%, 4%, 2%, 1%, 0.5%, 0.25%

Reaction mix preparation

5 Thermo Scientific™ Low Protein Binding Collection Tubes (1.5 mL) **Catalog #**PI90411

Calculate the reaction mix: [M] 2 mg/mL concentration of α-synuclein in 50 µL in reaction mix
Use protein low-binding tubes:

Reaction mix: 25 uL of chemical solution + 5 uL of PBS + 20 uL of 5 mg/mL sonicated fibrils
Once added, mix up very well
Prepare reaction mixes for each concentration of chemical solution in triplicates

6 Incubate at room temperature for 30 min

7 Evaluate the denaturation process using DLS analysis

Protocol

NAME

Dynamic Light Scattering measurements

CREATED BY

andrew.west west

Preview

8 Dilute samples up to 200 ng/mL (10.000x) to reach 1 mL of the volume, vortex before each dilution cycle

9 Follow instruction for the slot-blot analysis,
use Alexa-647-MJFR14-6-4-2 **Abcam Catalog #**#ab216309 at concentration 1:5000 to visualize aggregated forms of a-synuclein species after treatment with chemical compounds

Protocol

NAME

Slot-blot analysis of recombinant α-synuclein fibrils

CREATED BY

Arpine Sokratian

Preview