Aug 05, 2024

Slot-blot analysis of recombinant α-synuclein fibrils

DOI
dx.doi.org/10.17504/protocols.io.q26g7pkrkgwz/v1

Arpine Sokratian[1]

[1]Duke Univeristy

ASAP Collaborative Rese… West lab protocols

Arpine Sokratian
Duke Univeristy

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.q26g7pkrkgwz/v1

Protocol Citation: Arpine Sokratian 2024. Slot-blot analysis of recombinant α-synuclein fibrils. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7pkrkgwz/v1

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

Created: January 23, 2024

Last Modified: August 05, 2024

Protocol Integer ID: 93931

Keywords: ASAPCRN

Abstract

This protocol aims to provide an accurate analysis of aggregated species of α-synuclein. The method involves the immunoblotting of protein samples via low vacuum-induced filtration, followed by the blocking of the membrane and the attachment of antibodies specific to the protein. The procedure is designed to visualize the protein of interest in a slot-blot manner, which is suitable for intensity calculations. A critical aspect of this protocol is the correct setup of the slot-blot instrument to ensure equivalent vacuum pressure in each well of a 48-well setup. This step is crucial to guarantee the reproducibility and reliability of the results. The protocol is used to visualize and calculate the α-synuclein fibrils from cell tissues or those that are recombinantly expressed. Notably, the proteins are not denatured during the process, allowing for their capture in a physiological state

Protocol materials

Nonfat dry milk **Bio-Rad Laboratories** **Catalog #**1706404XTU Step 8

Recombinant Alexa Fluor® 647 Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] **Abcam** **Catalog #**ab216309

Step 9

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)

Sample preparation

1 5 mg/mL of sonicated fibrils (QC by DLS and nanodrop) were prepared in PBS
Chemical denaturants

	A	B
	SDS	10%
	Guanidine HCL	6M
	Sarkosyl	10%

Stock solutions

2 Prepare solutions of sonicated fibrils mixed with desired concentration of the denaturant, for example: 0.1 % SDS in 0,5 mg/mL sonicated fibrils solution, 0.01% SDS, 0.001% and etc.

3 Incubate solutions for 30 minutes (along with control samples prepared in PBS) at room temperature

STEP CASE

DLS of chemically denatured sonicated fibrils 8 steps

Measure DLS profiles of each samples, acq. time - 10 sec, number of acq.: 30

4 Dilute samples to the final concentration of fibrils - 1 ug/mL.

Bio-dot slot format microfiltration apparatut assembly

5 Clean and dry the Bio-Dot apparatus and gasket prior to assembly

Equipment

Bio-Dot Microfiltration Apparatus	NAME
Microfiltration Apparatus	TYPE
Bio-rad	BRAND
1703938	SKU

6 Insert previously soaked in TBS 0.22um nitrocellulose membrane using a bio-dot slot format microfiltration apparatus

Equipment

Bio-Dot® SF Microfiltration Apparatus	NAME
blotting apparatus	TYPE
Bio-Rad	BRAND
170-6542	SKU

7 Add 200 ul of TBS to 48 chambers in the apparatus. Apply the low-speed vacuum to filter the liquid through the membrane.
Add 200 ul of the fibril samples, the blank well should be filled with TBS, then apply the vacuum to fully drain the membrane.

Immunnoblotting

8 Place the membrane into the rack with 20 mL of TBS with 5% non-fat milk

Nonfat dry milk **Bio-Rad Laboratories Catalog #**1706404XTU

Block for 1 hour with continuous shaking at room temperature200 ul of samples in TBST.

9 Add the desired antibodies. In case to detect fibrilllar a-syn species add 1:5000 in TBST

Recombinant Alexa Fluor® 647 Anti-Alpha-synuclein aggregate antibody [MJFR-14-6-4-2] **Abcam Catalog #**ab216309

Incubate for 2 hours

10 Wash with TBST three times

11 Image Blot on BioRad Chemidoc

Equipment

ChemiDoc™ MP Imaging System	NAME
Imaging System	TYPE
Bio-rad	BRAND
12003154	SKU