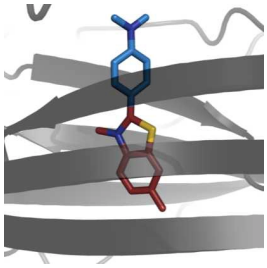
Aug 05, 2024

Amyloid dye-binding analysis of α-synuclein fibrils



DOI
dx.doi.org/10.17504/protocols.io.x54v9yznpg3e/v1

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DOI: dx.doi.org/10.17504/protocols.io.x54v9yznpg3e/v1

Protocol Citation: Arpine Sokratian 2024. Amyloid dye-binding analysis of α-synuclein fibrils. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9yznpg3e/v1

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

Created: June 03, 2022

Last Modified: August 05, 2024

Protocol Integer ID: 63822

Keywords: ASAPCRN

Funders Acknowledgement:
ASAP
Grant ID: 020527

Abstract

This protocol describes the amyloid binding assay used to capture the conformational signatures in expanded cohort of a-synuclein fibrils. Specific amyloid dyes indicated in the protocol display a distinct binding affinity to a-synuclein fibril variants and expose no signal in presence of native or unfolded protein

Protocol materials

Nile Red **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**19123-10MG Step 1

NIAD-4 2-[[5-(4-hydroxyphenyl)[22-bithiophen]-5-yl]-methylene]-propanedinitrile **caymanchem Catalog #**18520 Step 1

Thioflavin T **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T3516 Step 1

FSB **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**344101-5MG Step 1

Corning® 384-well Black/Clear Bottom Low Flange Ultra-Low Attachment Microplate Bulk Packed **Corning Catalog #**4588 Step 7

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)

Dye preparation

1 Prepare / thaw down the dye powder at Room temperature
Nile Red **Sigma Aldrich Catalog #**19123-10MG ,
NIAD-4 2-[[5-(4-hydroxyphenyl)[22-bithiophen]-5-yl]-methylene]-propanedinitrile **caymanchem Catalog #**18520
, Thioflavin T **Sigma Aldrich Catalog #**T3516
FSB **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**344101-5MG

2 Dissolve the dyes in DMSO to final concentration [M] 15 millimolar (mM) for NIAD-4, Nile Red and FSB,
Dissolve ThT in ddH2O to final concentration [M] 1 millimolar (mM)
Store aliquots at -80 °C

3 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

4 Evaluated sonicated fibrils should be diluted in PBS to concentration of 2 mg/mL.
Prepare serial dilutions with dilution factor of 2 in PBS.

5 Once dilutions of fibrils are prepared, thaw down the frozen aliquots of amyloid dyes in water-bath set at 42 °C

6 Dilute amyloid dyes in PBS to 100 uM concentration (volume 2-3 mL in 15 mL falcon tubes). IMPORTANT: Dyes need to be diluted after all other reagents are ready

7 Add 5 ul of dyes for each replicate/ dilution accounted for

Corning® 384-well Black/Clear Bottom Low Flange Ultra-Low Attachment Microplate Bulk Packed **Corning Catalog #**4588

IMPORTANT: Spin down the covered 384-well plate loaded with the dyes before moving on to the next step.

8 Once 5 uL of diluted dyes have been added to the 384-well plate, add the fibril/protein to the dye-preloaded wells. As a standard curve, we optionally use BSA fibrils or standard recombinant sonicated a-synuclein fibrils (starting from 5 mg/mL concentration)

9 Cover the plate with a sealing foil. Spin down the plate at 500g for 30 sec.

10 Set up the program in

Equipment

Microplate Reader

NAME

BMG Labtech CLARIOstar

BRAND

None

SKU

https://www.bmglabtech.com/clariostar-plus/

LINK

11 The program should include the incubation period of 5 min at continuous shaking at 200 rpm. Fluorescence intensity is recorded with excitation/emission spectra set at 468-15/510-20 for FBS, 535-20/585-30 for nile red and 448-10/482-10 nm for ThT. Monomeric mouse and human a-synuclein monomers should be used as a controls (include blank well with PBS as well).

12 Extrapolate the results by subtracting the average blank value. Then, correlate these adjusted results with the standard curve. Ensure that the extrapolated values fall within the appropriate range of the standard curve.