

May 14, 2024

## Aggregated aSyn Dot Blot assay

 Forked from [Aggregated aSyn Dot Blot assay](#)

DOI

**[dx.doi.org/10.17504/protocols.io.bp2l629x1gqe/v1](https://dx.doi.org/10.17504/protocols.io.bp2l629x1gqe/v1)**

rabdelha<sup>1</sup>

<sup>1</sup>California Institute of Technology

ASAP Collaborative Rese...



**gustavo.parfitt** Parfitt

University College of London

OPEN  ACCESS



DOI: [dx.doi.org/10.17504/protocols.io.bp2l629x1gqe/v1](https://dx.doi.org/10.17504/protocols.io.bp2l629x1gqe/v1)

External link: <https://doi.org/10.7554/eLife.81453>

**Protocol Citation:** rabdelha 2024. Aggregated aSyn Dot Blot assay. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.bp2l629x1gqe/v1>

### Manuscript citation:

Abdel-Haq R, Schlachetzki JC, Boktor JC, Cantu-Jungles TM, Thron T, Zhang M, Bostick JW, Khazaei T, Chilakala S, Morais LH, Humphrey G, Keshavarzian A, Katz JE, Thomson M, Knight R, Gradinaru V, Hamaker BR, Glass CK, Mazmanian SK, A prebiotic diet modulates microglial states and motor deficits in  $\alpha$ -synuclein overexpressing mice. eLife doi: [10.7554/eLife.81453](https://doi.org/10.7554/eLife.81453)

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Working

**We use this protocol and it's working**

**Created:** May 12, 2022

**Last Modified:** May 14, 2024

**Protocol Integer ID:** 99796



**Keywords:** Aggregated aSyn Dot Blot assay, Proteinase K resistant aSyn histology, ASAPCRN

## Abstract

This protocol details aggregated aSyn Dot Blot assay.

## Attachments

















**432-920.docx**

94KB



## Aggregated aSyn Dot Blot assay

- 1 Normalize protein samples to equal concentrations between  100 ng -  1000 undetermined in PBS.
- 2 Carefully spot  1.5  $\mu$ L each sample onto dry nitrocellulose (  0.45  $\mu$ m pores) membrane. 
- 3 Allow moisture to dry for a minute or two.
- 4 Block membrane in 5% skim milk in TBST.
- 5 Wash membrane 1X in TBST. 
- 6 Apply anti-aggregated aSyn antibody in 5% skim milk in TBST (1:1000 for Abcam fibril.aggregate specific aSyn antibody: MJFR 14-6-4-2).
- 7 Incubate at  Room temperature for 1-2 hours, or  Overnight at  4 °C .   
 
- 8 Wash 3x with TBST. 
- 8.1 Wash with TBST (1/3).
- 8.2 Wash with TBST (2/3).
- 8.3 Wash with TBST (3/3).
- 9 Incubate with anti-rabbit IgG (or appropriate secondary) at 1:1000 for 1-2 hours in TBST. 



10 Wash 3x with TBST.



10.1 Wash with TBST (1/3).

10.2 Wash with TBST (2/3).

10.3 Wash with TBST (3/3).

11 Develop with Biorad Chemiluminescent substrate, or similar.

## Proteinase K resistant aSyn histology

1h 10m

12



### Note

Certainly can be modified for either DAB or Fluorescent staining, I have DAB protocol too

Wash 50um brain sections 1x in PBS.

13 Treat with Protinase K (NEB, 1:4000, stock at [M] 20 mg/mL ) for 00:10:00 at Room temperature .

10m

14 Wash 2x with IHC buffer (3% NGS, 0.3% TX100, in PBS).



14.1 Wash with IHC buffer (3% NGS, 0.3% TX100, in PBS) (1/2).

14.2 Wash with IHC buffer (3% NGS, 0.3% TX100, in PBS) (2/2).



15 Block with Mouse-on-Mouse kit (Vector Lab, PK-2200, just first step in instructions) for

01:00:00 .

1h

16 Wash 2x with IHC buffer.



16.1 Wash with IHC buffer (1/2).

16.2 Wash with IHC buffer (2/2).

17 Incubate Overnight at 4 °C with primary antibody 1:250 – 1:1000 (anti-synuclein BD Biosci 610787).



18 Wash 3x with PBS.



18.1 Wash with PBS (1/3).

18.2 Wash with PBS (2/3).

18.3 Wash with PBS (3/3).

19 Incubate with desired secondary (anti-Mouse IgG AF488 is my choice at 1:1000).



20 Wash 3x with PBS.



20.1 Wash with PBS (1/3).

20.2 Wash with PBS (2/3).



20.3 Wash with PBS (3/3).

21 Stain with Nissl and Mount with Profade with DAPI.