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Aggregated aSyn Dot Blot assay



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Protocol status: Working

We use this protocol and it's working

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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 μ L in PBS.
- 2 Carefully spot  1.5 μ L each sample onto dry nitrocellulose ( 0.45 μ 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 $^{\circ}$ C .

1h

 


- 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 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.