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Aggregated aSyn Dot Blot assay

rabdelha [1]

[1]California Institute of Technology

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rabdelha

This protocol details aggregated aSyn Dot Blot assay.

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Aggregated aSyn Dot Blot assay, Proteinase K resistant aSyn histology

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Aggregated aSyn Dot Blot assay

1 Normalize protein samples to equal concentrations between **100 ng** - **1000 ng/µl** in PBS.

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2 

Carefully spot **1.5 µL** each sample onto dry nitrocellulose (**0.45 µm** 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 

1h

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).

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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

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

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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).

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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.

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