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We use this protocol and it's working

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Immunohistochemistry using paraffin embedded tissue

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ABSTRACT

This protocol describes how to stain paraffin wax embedded sections of tissue for alpha-synuclein by immunofluorescence or DAB/peroxidase immunohistochemistry and is based on Hurley et al., 2013 (https://doi.org/10.1093/brain/awt134).

The protocol works for many other antibodies and the only change necessary for most antibodies is determining the optimal dilution of the primary antibody and the best type of antigen-retrieval to use.

MATERIALS

Rabbit anti-alpha-synuclein ab212184 Abcam

Goat anti-rabbit IgG biotin111-065-144-JIR Stratech

Goat anti-rabbit IgG Alexa Fluor™ 488 A27034 Thermo Fisher

Streptavidin POD11089153001

Wax embedding

1 Dissect tissue (fresh or trans-cardially perfused with formalin (10% neutral buffered formaldehyde) or 4% paraformaldehyde in PBS).

Safety information

Formaldehyde is toxic by inhalation
HT501128 (sigmaaldrich.com)

2 Place tissue in at least 5 volumes of fixative at 4 °C for > 48:00:00 if tissue was perfused or > 96:00:00 if fresh. The tissue can stay in fixative indefinitely. If the tissue was fresh and the solution is turbid with blood, change the fixative after 24 hours. 6d

3 Embed formalin fixed tissue in wax using an automated tissue processor. It can be done manually if you do not have access to an automated tissue processor. Histo-Clear™ II can be used instead of xylene.

Safety information

Xylene is toxic by inhalation and flammable
Xylene - Safety Data Sheet (chemicalbook.com)

Ethanol is toxic inhalation and flammable
Ethanol - Safety Data Sheet (chemicalbook.com)

3.1 **Gut tissue** 13h 42m

1.	10 % formalin	00:01:00	Room temperature
2.	10 % formalin	00:01:00	Room temperature
3.	70 % ethanol	01:00:00	30 °C
4.	90 % ethanol	01:00:00	30 °C
5.	100 % ethanol	01:00:00	30 °C
6.	100 % ethanol	01:00:00	30 °C
7.	100 % ethanol	01:00:00	30 °C
8.	100 % ethanol	01:20:00	30 °C

9. xylene 01:00:00 30 °C
10. xylene 01:00:00 30 °C
11. xylene 01:20:00 30 °C
12. paraffin wax 01:20:00 62 °C
13. paraffin wax 01:20:00 62 °C
14. paraffin wax 01:20:00 62 °C

3.2 **Brain tissue** (whole mouse)

1d 2h 2m

1. 10 % formalin 00:01:00 Room temperature
2. 10% formalin 00:01:00 Room temperature
3. 70% ethanol 02:00:00 30 °C
4. 90% ethanol 02:00:00 30 °C
5. 100% ethanol 02:00:00 30 °C
6. 100% ethanol 02:00:00 30 °C
7. 100% ethanol 02:00:00 30 °C
8. 100% ethanol 02:00:00 30 °C
9. xylene 02:00:00 30 °C
10. xylene 02:00:00 30 °C
11. xylene 04:00:00 30 °C
12. paraffin wax 02:00:00 62 °C
13. paraffin wax 02:00:00 62 °C
14. paraffin wax 02:00:00 62 °C

4 Mount the tissue in a block of wax using an embedding station and metal or plastic moulds.
Store at Room temperature

5 Section the tissue (5 µm to 8 µm)using a microtome.
Store sections at Room temperature

Dewaxing and antigen retrieval

6 Select slides to be stained and place in a microscope slide rack.

7 Heat sections 60 °C 00:30:00 30m

8 Dewax sections in two changes of Histo-Clear II (National Diagnostics). 20m
1. 00:10:00
2. 00:10:00

9 Rehydrate sections through graded (v/v) ethanol solutions. 25m
1. 100 % 00:05:00
2. 100 % 00:05:00
3. 95 % 00:05:00
4. 80 % 00:05:00
5. 70 % 00:05:00

10 Wash with PBS 00:05:00 5m

11 Quench endogenous peroxidase with 0.3 % hydrogen peroxide (v/v) 00:05:00 5m

12 Wash with PBS 00:05:00 5m

13 For antigen retrieval heat 100 °C the sections in 15 mM sodium citrate buffer containing 0.1% Tween® 20, pH 6 for 00:25:00 in an 800-watt microwave set on 30 % power. 25m

14 Cool with running tap water 00:05:00 5m

15 Wash with PBS **2** x 00:05:00 5m

16 For **immunofluorescence** staining go to step **17**

For **DAB/peroxidase** staining go to step **27**

Immunofluorescence staining 23h 25m

17 Draw around the sections with a wax pen.

18 Block non-specific binding sites with 10 % goat serum in PBS containing 0.005 % Triton™ X-100 and 0.05 % thimerosal 01:30:00 1h 30m

Depending on the size and number of sections 150-250 microliters of solution should be sufficient to cover the sections.

19 Incubate with anti-alpha-synuclein antibody (1:500) (ab212184) in 10 % goat serum containing 0.005 % Triton™ X-100 and 0.05 % thimerosal (antibody buffer) 20:00:00 20h

Room temperature

20 Wash with PBS 4 x 00:05:00 5m

21 incubated sections with Alexa Fluor goat anti-rabbit IgG (1:200) (A27034) 01:30:00 1h 30m

22 Wash with PBS **2** x 00:05:00 5m

23 incubate sections with DAPI (1 µg/ml) 00:05:00 5m

24 Wash with PBS **3** x 00:05:00 5m

25 Dip slides in water to remove salts

26 Mount sections using Hydromount™ (National Diagnostics).

DAB/peroxidase staining 43m 15s

27 Draw around the sections with a wax pen.

28 Block non-specific binding sites with 10 % goat serum in PBS containing 0.005 % Triton™ X-100 and 0.05 % thimerosal 01:30:00 1h 30m

Depending on the size and number of sections 150-250 microliters of solution should be sufficient to cover the sections.

29 Incubate with anti-alpha-synuclein antibody (1:500) (ab212184) in 10 % goat serum containing 0.005 % Triton™ X-100 and 0.05 % thimerosal (antibody buffer) 20:00:00 20h

Room temperature

30 Wash PBS **4** x 00:05:00 5m

31 Incubate sections in biotinylated goat anti-rabbit IgG (1:250) secondary antibody for 2 hours.

32 Wash PBS **4** x 00:05:00 5m

33 Incubate sections with streptavidin-peroxidase conjugate (1:250) for 1 hour.

34 Wash PBS **4** x 00:05:00 5m

35 Visualise staining by incubation with PBS containing 0.05 % (w/v) 3,3'-Diaminobenzidine/0.015 % (v/v) H_2O_2/0.05 % (w/v) nickel ammonium sulphate for up to 00:03:00 , 3m

36 Wash sections for > 00:05:00 in running tap water. 5m

37 Counter stain with haematoxylin 00:00:05 to 00:00:10 if desired. 15s

38 Wash sections with running tap water until it runs clear to remove excess stain.

39 Dehydrate sections through graded (v/v) ethanol solutions 25m

70 % 00:05:00

80 % 00:05:00

95 % 00:05:00

100 % 00:05:00

100 % 00:05:00

40 Clear sections with Histo-Clear II (National Diagnostics) **2** x 00:05:00 5m

41 Mount with Omnimount™ (National Diagnostics) or equivalent permanent resin.