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Protocol status: Working 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)

- Place tissue in at least 5 volumes of fixative at $4 \, ^{\circ}\text{C}$ 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.
- 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

1. 10 % formalin

2. 10 % formalin

3. 70 % ethanol

4. 90 % ethanol

5. 100 % ethanol

6. 100 % ethanol

7. 100 % ethanol

8. 100 % ethanol

© 00:01:00 Room temperature

© 00:01:00 BRoom temperature

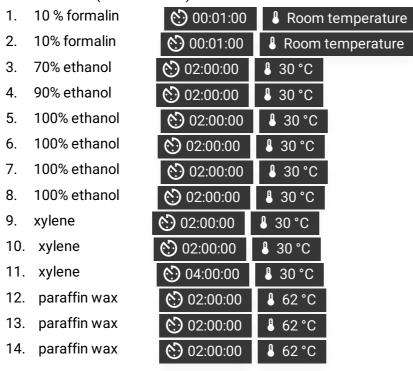
 6d

13h 42m

9.	xylene	© 01:00:00	3 0 °C
10.	xylene	(5) 01:00:00	1 30 °C
11.	xylene	(5) 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



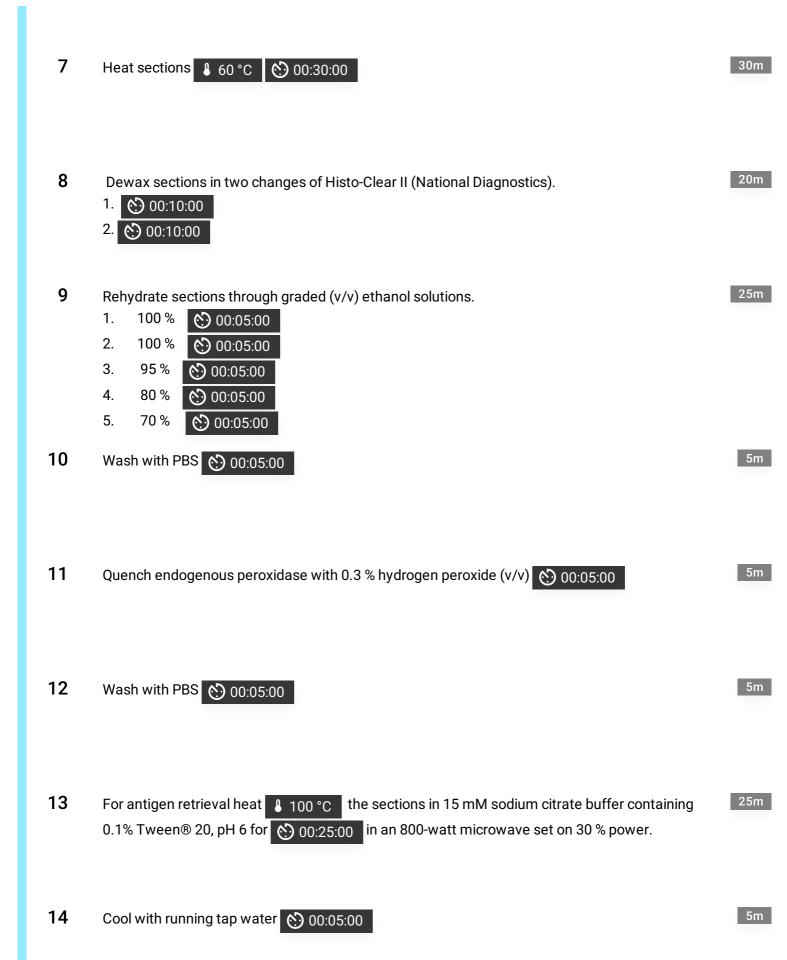
4 Mount the tissue in a block of wax using an embedding station and metal or plastic moulds.

Store at 8 Room temperature

Section the tissue (\rightarrow | + 5 μ m to \rightarrow | + 8 μ m) using a microtome. Store sections at 8 Room temperature

Dewaxing and antigen retrieval

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



15 Wash with PBS **2** x (5) 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 1h 30m Block non-specific binding sites with 10 % goat serum in PBS containing 0.005 % Triton™ X-100 and 0.05 % thimerosal (5) 01:30:00 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

Room temperature

20h

20 Wash with PBS 4 x 00:05:00

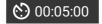
5m

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

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



5m

24 Wash with PBS **3** x **6** 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 (5) 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

- 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

- Incubate sections with streptavidin-peroxidase conjugate (1:250) for 1 hour.
- 34 Wash PBS 4 x 🕙 00:05:00

5m

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 $O_2/0.03:00$,

3m

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

5m

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

15s

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

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



- 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.