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# Immunohistochemistry Protocol (brain post-mortem tissue)

Forked from a private protocol


DOI
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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol details the immunohistochemistry steps for antibody blocking over a period of 2 days.

## Attachments

PDF

Immunohistochemistry..

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107KB

## Materials

### REAGENTS

- Xylene
- 70% EtOH
- 90% EtOH
- 100% EtOH
- $dH_2O$
- Sodium citrate buffer: 2.94g Tri-sodium citrate (dehydrate), 1000ml Distilled Water (adjust to pH 6 with 1N HCl) then add 0.5ml Tween 20
- TBPS: 1x PBS + 0.05% Tween 20
- 3% $H_2O_2$ diluted in TPBS
- Blocking solution (e.g. 5% Normal horse serum in TPBS)
- Primary and Secondary Antibody
- ABC solution (DAB substrate): 5ml TPBS with 2 drops of reagent A and 2 drops of reagent B
- DAB stain: 5 ml $dH_20$ with: 2 drops of buffer (pH 7.5), 4 drops of ABC and 2 drops of hydrogen peroxide
- Hematoxylin
- 1% Acid alcohol: 350ml industrial methylated spirit (denatured alcohol), 5ml HCl, 145ml $dH_2O$
- Mounting medium: DPX

### CONSUMABLES

- Slides
- Tip box for hematoxylin

### EQUIPMENT

- Microwave

## Safety warnings

For safety warnings and hazards, see the Safety Data Sheet (SDS).

## Day 1

5m

1 **Sequential rehydration**
Xylene, 100% EtOH, 90% EtOH, 70% EtOH, $dH_2O$ – 5 min in each:

1.1 Rehydrate samples in Xylene for 00:05:00 . 5m

1.2 Rehydrate samples in 100% EtOH for 00:05:00 . 5m

1.3 Rehydrate samples in 90% EtOH for 00:05:00 . 5m

1.4 Rehydrate samples in 70% EtOH for 00:05:00 . 5m

1.5 Rehydrate samples in $dH_2O$ for 00:05:00 . 5m

2 **Antigen Retrieval** 20m
Microwave slides at high power for 00:20:00 in
[M] 0.05 % (v/v) Tween 20 in Sodium Citrate Buffer , pH 6 .

Note

Sodium citrate recipe:
2.94 g Tri-sodium citrate (dehydrate)
1000 mL Distilled water
Mix to dissolve, adjust to pH 6 with 1N HCl and then add 0.5 mL Tween 20 and mix well.

*Store at RT for 3 months or at 4°C for longer storage.

3 **Wash**
3 × 5 min with TBPS ( [M] 0.05 % (v/v) Tween 20 in 1xPBS ):

3.1 Wash 1 of 3: 00:05:00 with TBPS (1x PBS + 0.05% Tween 20) 5m

3.2 Wash 2 of 3: 00:05:00 with TBPS (1x PBS + 0.05% Tween 20) 5m

3.3 Wash 3 of 3: 00:05:00 with TBPS (1x PBS + 0.05% Tween 20) 5m

4 **Endogenous peroxidase blocking** 15m

Incubate slides in [M] 3 % (v/v) hydrogen peroxide (H2O2) in TPBS at Room temperature for 00:15:00 .

5 **Wash**

3 × 5 min with TBPS:

5.1 Wash 1 of 3: 00:05:00 with TBPS 5m

5.2 Wash 2 of 3: 00:05:00 with TBPS 5m

5.3 Wash 3 of 3: 00:05:00 with TBPS 5m

6 **Blocking for 1 hour**

Blocking Solution (e.g. 5% Normal horse serum in TPBS).

7 **Primary antibody – Overnight incubation** 15m

Dilute in TPBS in appropriate concentration and incubate Overnight at 4 °C .

## Day 2 4h 3m 31s

8 **Wash**

3 × 5 min with TPBS:

8.1 Wash 1 of 3: 00:05:00 with TBPS 5m

8.2 Wash 2 of 3: 00:05:00 with TBPS 5m

8.3 Wash 3 of 3: 00:05:00 with TBPS 5m

9 **Secondary antibody** 2h
Dilute antibody in blocking solution and incubate for 02:00:00 at
Room temperature

10 **Wash**
3 × 5 min with 1xTPBS:

10.1 Wash 1 of 3: 00:05:00 with 1xTBPS 5m

10.2 Wash 2 of 3: 00:05:00 with 1xTBPS 5m

10.3 Wash 3 of 3: 00:05:00 with 1xTBPS 5m

11 **ABC solution (DAB substrate)** 1h

Note

*Prepare 00:30:00 before use!

Make 5 mL TPBS with 2 drops of reagent A and 2 drops of reagent B. Leave ABC on tissue for 01:00:00

12 **Wash**
3 × 5 min with TPBS:

12.1 Wash 1 of 3: 00:05:00 with TBPS 5m

12.2 Wash 2 of 3: 00:05:00 with TBPS 5m

12.3 Wash 3 of 3: 00:05:00 with TBPS 5m

13 **DAB Stain**

Make 5 mL dH20 with: 2 drops of buffer ( pH 7.5 ), 4 drops of ABC and 2 drops of hydrogen peroxide. Incubate with DAB at Room temperature until colour change.

14 **Wash**

2 × 5 min with $dH_20$:

14.1 Wash 1 of 2: 00:05:00 with dH20 5m

14.2 Wash 2 of 2: 00:05:00 with dH20 5m

15 **Counterstaining with Hematoxylin** 30s

Place slides in tip box of hematoxylin for 00:00:30 .

15.1 1 x 00:05:00 wash with $dH_20$. 5m

15.2 Put slides in acid alcohol, 00:00:01 quickly in and out. Keep the slides in running water for 00:03:00 for blueing. 3m 1s

Note

**1% Acid alcohol:**

350 mL Industrial methylated spirit (denatured alcohol)

5 mL HCl

154 mL dH2O

16 **Dehydration process**

70% EtOH, 90% EtOH, 100% EtOH, Xylene:

16.1 Dehydrate samples in 70% EtOH.

16.2 Dehydrate samples in 90% EtOH.

16.3 Dehydrate samples in 100% EtOH.

16.4 Dehydrate samples in Xylene.

17 **Mounting Slides**
Mount slides using medium DPX.