



Feb 20, 2025

🌐 IBA1 Immunohistochemistry on human paraffin sections



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DOI

<https://dx.doi.org/10.17504/protocols.io.ewov1dwo7vr2/v1>

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Protocol Citation: Marta Gonzalez-Sepulveda, Joan Compte, Miquel Vila 2025. IBA1 Immunohistochemistry on human paraffin sections. [protocols.io https://dx.doi.org/10.17504/protocols.io.ewov1dwo7vr2/v1](https://dx.doi.org/10.17504/protocols.io.ewov1dwo7vr2/v1)

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Protocol status: Working

We use this protocol and it's working

Created: February 19, 2025

Last Modified: February 20, 2025

Protocol Integer ID: 121669

Keywords: iba1 immunohistochemistry on human paraffin sections iba1, human paraffin sections iba1, iba1 immunohistochemistry, specific immunohistochemistry protocol on paraffin, embedded human brain section, human brain section, specific immunohistochemistry protocol, paraffin

Abstract

Iba1-specific Immunohistochemistry protocol on paraffin-embedded human brain sections

Materials

Reagents :

- TBS 10X : Tris base 121.1g + NaCl 90g in 1L H₂O.pH 7.4.
- TBS 1X-Triton 0,5%
- Xilen
- Ethanol : 100%, 95%, 70%
- Unmasking buffer epitopes : Citrate solution 10mM pH6.0
- Blocking Buffer : TBS 1X + 5% NGS
- 1st Ab : Diluted in1X PBS +2%NGS
- 2nd Ab : Diluted in1X PBS +2%NGS
- Endogenous peroxidase blocking solution : TBS 1x + 3% H₂O₂(30%) + 10% methanol



1. Deparaffinization and hydratation :

- 1 Put the slides 30 min in the incubator at 60°C.
- 2 Wash 3×3 min in Xilen.
- 3 Wash 1×10 min in ethanol 100%.
- 4 Wash 1×10 min in ethanol 95%.
- 5 Wash 1×5 min in ethanol 70%.
- 6 Wash 2×5 min in TBS 1X.

2. Blocking endogenous peroxidase:

- 7 Put the slides 10 min in endogenous peroxidase blocking solution: TBS 1x + 3% H₂O₂ + 10% methanol

Wahsing

- 8 Wash 3×5 min in buffer TBS 1X.

Antigen retrieval

- 9 Put sections in 200 mL of citrate buffer 10mM, pH 6
- 9.1 Put sections in a boiling water bath for 20 min



- 10 Let sections cool down for 20 min at RT

Washing

- 11 Wash 3×5 min in TBS 1X.

Blocking

- 12 Put wet paper in a black box for immunohistochemistry
- 13 Gently dry and circle sections with the hydrophobic pen ImmEdge Vector H 4000 without touching the tissue
- 14 Blocking in TBS 1X + 5% NGS (200uL/slides) 1h at RT

1ary antibody

- 15 Gently wipe off the water of the slides
- 16 Put 200ul/slide of TBS 1X + 2% NGS + anti-IBA1 primary antibody at a concentration of 1:500(Abcam, #ab178846) at 4°C (cold room)

Washing

- 17 Wash 3×5min in TBS 1X Buffer.

2ary antibody

- 18 Gently wipe off the water of the slides
- 19 Put 200ul/slide TBS 1X + 2% NGS + biotinylated secondary antibody



at a concentration of 1:1000 (BA-1000-1.5, Palex) 1h at RT.

- 19.1 At this step it is important to prepare ABC solution and let it, at least, 30 min on the shaker

Washing

- 20 3×5min in TBS 1X

Peroxidase staining

- 21 Incubate 1 hour at RT with ABC solution (4drops A/B in 10 mL of TBS 1x)

Washing

- 22 3×5min in TBS 1X

Developing preparation

- 23 In aluminium foil: Vector SG: 3 drops Chromogen + 3 drops Hydrogen Peroxide in 5mL PBS (gives rise to blue staining)

Developing

- 24 Cover tray plates with aluminium foil
- 24.1 Put 200 uL on each section and put a cardboard box on it to keep darkness for a time ranging from 3-15 minutes depending on the antibody used
- 24.2 Remove with an air-pump equipment and clean the material with bleach

Washing

- 25 3×5min in TBS 1X



Dehydration

- 26 1 min in ethanol 70%
- 27 1 min in ethanol 95%
- 28 1 min in ethanol 100%.
- 29 2×5 min in xylene

Mounting

- 30 Put a line of mounting medium(DPX) byslide.Put the coverslip (washed with ethanol previously) on the slide. Remove bubbles
- 31 Let dry the slides on the tray in the hood overnight