

Sep 12, 2022

Immunochemistry on paraffin sections

 In 2 collections

DOI

<https://dx.doi.org/10.17504/protocols.io.kxygx9my4g8j/v1>

Marta Gonzalez-Sepulveda¹, Joan Compte¹, Miquel Vila¹

¹Vall d'Hebron Research Institute

Vilalab Public



Miquel Vila

VHIR-CIBERNED-ASAP

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kxygx9my4g8j/v1>

Protocol Citation: Marta Gonzalez-Sepulveda, Joan Compte, Miquel Vila 2022. Immunochemistry on paraffin sections. protocols.io <https://dx.doi.org/10.17504/protocols.io.kxygx9my4g8j/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: September 12, 2022

Last Modified: November 10, 2023

Protocol Integer ID: 69880

Keywords: immunochemistry on paraffin sections immunochemistry protocol, paraffin sections immunochemistry protocol, embedded rat brain section, rat brain section, paraffin, immunochemistry, brain

Abstract

Immunochemistry protocol on paraffin-embedded rat 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 hydration :

- 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 + primary Ab48/72h (it depends of the Ab) 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 + Secondary Ab 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: DAB Standard Kit: 1 drop of reagent B in 1 mL of reagent A (gives rise to brown staining)
- 23.1 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) by slide. Put the coverslip (washed with ethanol previously) on the slide. Remove bubbles

31 Let dry the slides on the tray in the hood overnight