Nov 09, 2019

General Immunohistochemical Protocol for formalin-fixed, paraffin-embedded tissue sections

Forked from Immunohistochemistry Protocol for Paraffin-Embedded Sections

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.7uxhnxn

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EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0224818

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Blassova T, Tonar Z, Tomasek P, Hosek P, Hollan I, Treska V, Molacek J (2019) Inflammatory cell infiltrates, hypoxia, vascularization, pentraxin 3 and osteoprotegerin in abdominal aortic aneurysms – A quantitative histological study. PLoS ONE 14(11): e0224818. doi: 10.1371/journal.pone.0224818

SAFETY WARNINGS

Formalin is a suspect carcinogen. It can cause eye, skin, and respiratory tract irritation. It should be handled in a hood.

DAB is a suspect carcinogen. Handle with care. Wear gloves, lab coat and eye protection.

BEFORE STARTING

- For initial experiments, the user must titrate primary and secondary reagents so that staining with the secondary antibody alone yields no background while staining with primary and secondary antibodies yields strong, specific staining.
- Take care to ensure that slides do not dry out by incubating with sufficient volumes and/or in a humidified chamber (such as 926301).

General Immunohistochemical Protocol for formalin-fixed, paraffin-embedded tissue sections

1 Deparaffinize slides in 3 changes of xylene, 5 min each.
00:05:00

2 Transfer slides to 100% alcohol, for 2 changes, 3 min each, and then transfer once through 95%, 70% and 50% alcohols respectively for 3 min each. 00:05:00

3 Block endogenous peroxidase activity by incubating sections in Peroxidase-Blocking solution (Dako) at room temperature for 10 min to block endogenous peroxidase activity.
00:10:00

4 Rinse in 300ml of PBS for 3 changes, 5 min each.
00:05:00

5 *Optional:* Perform antigen retrieval to unmask the antigenic epitope. The most commonly used antigen retrieval is a citrate buffer method. Arrange the slides in a staining container. Pour 300ml of 10mM citrate buffer, pH 6.0 into the staining container and incubate it at 95-100°C for 10 min (optimal incubation time should be determined by user). Remove the staining container to room temperature and allow the slides to cool for 20 min.

We used different ways depend to used primary antibody: - Heat-induced epitope retrieval in Epitope Retrieval Solution pH 9 X pH 6 (Novocastra Leica, Leica Biosystems GmbH, Nussloch, Germany); 20 minutes
- Proteinase K (Dako) - 10 min.

6 Rinse slides in 300ml PBS for 3 changes, 5 min each. 00:05:00

7 *Optional:* Add 200µl blocking buffer (e.g. 10% goat serum in PBS) onto the sections of the slides and incubate in a humidified chamber at room temperature for 1 hour. 00:30:00

8 Drain off the blocking buffer from the slides.

9 Apply 200µl appropriately diluted primary antibody (in antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temperature overnight. 16:00:00 **overnight**
4 °C

10 Wash the slides in 300ml PBS for 3 changes 5 min each.
00:05:00

11 Apply 200µl appropriately diluted secondary antibody (using the antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temperature for 30 min.

We used: N-Histofine® Simple Stain MAX PO (NICHIREI CORPORATION INC.)
Apply 2 drops of Simple Stain MAX PO (MULTI) to each slide so as to provide a complete cover of the sections. Incubate at room temperature for 30 minutes.
00:30:00

12 Wash slides in 300ml PBS for 3 changes, 5 min each.
00:05:00

13 Apply 150 µl DAB Liquid solution (Dako) to the sections on the slides to reveal the color of antibody staining. Allow the color development for < 5 min until the desired color intensity is reached.

(Caution: DAB is a suspect carcinogen. Handle with care. Wear gloves, lab coat and eye protection.)

14 Rinse slides in destil water, 5 min each.
00:05:00

15 *Optional:* Counterstain slides by immersing sides in Hematoxylin (e.g. Gill's Hematoxylin) for 1-2 min.
00:02:00

16 Rinse the slides in running tap water for > 15 min.
00:15:00

17 Dehydrate the tissue slides through 4 changes of alcohol (95%, 95%, 100% and 100%), 5 min each. **00:05:00**

18 Clear the tissue slides in 3 changes of xylene and coverslip using mounting solution. The mounted slides can be stored at room temperature permanently.

19 Observe the color of the antibody staining in the tissue sections under microscopy.