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Immunohistochemistry Protocol for Paraffin-Embedded Sections V.3

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1 Works for me dx.doi.org/10.17504/protocols.io.97bh9in

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EXTERNAL LINK

https://www.biolegend.com/protocols/immunohistochemistry-protocol-for-paraffin-embedded-sections/4256/

GUIDELINES

General Tips and FAQ:

FAQ:

Do I need to perform antigen retrieval on my formalin-fixed, paraffin-embedded samples prior to staining?

- In most cases, this is true. Antigen retrieval helps both the accessibility of the antibody to the tissue and also counteracts the fixation effects on the recognized epitopes. Check the application references for any additional details for IHC or IF experiments.

Can antibody X be used for immunohistochemistry? What concentration do I use?

- Typical concentrations of monoclonal antibodies for use in IHC are from 5-25 µg/ml. Polyclonal antibodies can be used at a range of 1-10 µg/ml. While we do not test for IHC application in house, we will indicate on the datasheet if an antibody has been published for use in this application. In addition, you can do a literature search with the clone name and immunohistochemistry/paraffin/frozen to see what the protocol details are.

Tips:

- 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).

Prepare formalin-fixed, paraffin-embedded tissue sections (steps 1-8):

1 Fix freshly dissected tissue (less than 3 mm thick) with 10% formalin or other fixatives for 24-48 hour at room temperature. Caution: Formalin is a suspected carcinogen. It can cause eye, skin, and respiratory tract irritation. It should be handled in a hood.

2 Rinse the tissue with running tap water for 1 hour.

3 Dehydrate the tissue through 70%, 80%, and 95% alcohol, 45 min each, followed by 3 changes of 100% alcohol, 1 hour each.

4 Clear the tissue through 2 changes of xylene, 1 hour each.

5 Immerse the tissue in 3 changes of paraffin, 1 hour each.

6 Embed the tissue in a paraffin block. The paraffin tissue block can be stored at room temperature for years.

7 Section the paraffin-embedded tissue block at 5-8 µm thickness on a microtome and float in a 40°C water bath containing distilled water.

8 Transfer the sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost Plus). Allow the slides to dry overnight and store slides at room temperature until ready for use.

Immunostain formalin-fixed, paraffin-embedded tissue sections (steps 9-29):

9 Deparaffinize slides in 2 changes of xylene, 5 min each.

10 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.

11 Block endogenous peroxidase activity by incubating sections in 3% H2O2 solution in methanol at room temperature for 10 min to block endogenous peroxidase activity.

12 Rinse in 300 ml of PBS for 2 changes, 5 min each.

13 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 300 ml of 10 mM 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.

14 Rinse slides in 300 ml PBS for 2 changes, 5 min each.

15 Optional: Add 100 µl blocking buffer (e.g. 10% fetal bovine serum in PBS) onto the sections of the slides and incubate in a humidified chamber at room temperature for 1 hour.

16 Drain off the blocking buffer from the slides.

17 Apply 100 µl appropriately diluted primary antibody (in antibody dilution buffer, e.g. 0.5% bovine serum albumin in PBS) to the sections on the slides and incubate in a humidified chamber at room temperature for 1 hour.

18 Wash the slides in 300 ml PBS for 2 changes 5 min each.

19 Apply 100 µl appropriately diluted biotinylated 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.

20 Wash slides in 300ml PBS for 2 changes, 5 min each.

21 Apply 100 µl appropriately diluted Sav-HRP conjugates (using the antibody dilution buffer) to the sections on the slides and incubate in a humidified chamber at room temperature for 30 min (keep protected from light).

22 Wash slides in 300ml PBS for 2 changes, 5 min each.

23 Apply 100µl DAB substrate solution (freshly made just before use: 0.05% DAB - 0.015% H2O2 in PBS) to the sections on the slides to reveal the color of antibody staining. Allow the color development for less than 5 min until the desired color intensity is reached. Caution: DAB is a suspected carcinogen. Handle with care. Wear gloves, lab coat and eye protection.

24 Wash slides in 300 ml PBS for 3 changes 2 min each.

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

26 Rinse the slides in running tap water for more than 15 min.

27 Dehydrate the tissue slides through 4 changes of alcohol (95%, 95%, 100%, and 100%), 5 min each.

28 Clear the tissue slides in 3 changes of xylene and coverslip using mounting solution (e.g. Permount). The mounted slides can be stored at room temperature permanently.

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