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IMMUNOHISTOCHEMISTRY PARAFFIN-EMBEDDED TISSUE PROTOCOL (ENZYMATIC/CHROMOGENIC)

Preparing paraffin-embedded tissue sections

These steps are needed for clearing tissues from paraffin and rehydrating them.

1. Immerse slides with tissue sections into xylene: 3 changes, 10 minutes each.
2. Transfer slides from xylene into 100% ethanol: 3 changes, 10 minutes each.
3. Immerse slides into 95% ethanol: 1 change, 5 minutes.
4. Immerse slides into 70% ethanol: 1 change, 5 minutes.
5. Immerse slides into 50% ethanol: 1 change, 5 minutes.
6. Rinse slides with distilled water for 5 minutes.
7. Transfer slides into 1x wash buffer (OOMB00005).

Antigen Retrieval

This step is needed to unmask tissue antigens from chemical fixation of tissues.

1. Prepare working dilution of antigen retrieval solution (OOMB00006 or OOMB00007).
2. Fill slide holding jar with antigen retrieval solution and preheat to 95-98°C.
3. Immerse slides into jars with hot retrieval solution and place them into either a pressure cooker or vegetable steamer for 10-15 minutes.
4. Transfer jars with slides onto a lab bench and let them cool to room temperature for 25-30 minutes.
5. Rinse slides with distilled water and transfer them into 1x wash buffer (OOMB00005).

Now tissue sections are ready for IHC staining procedure. Incubations and washing steps are done at room temperature unless indicated otherwise.

For HRP staining

1. Place slides horizontally into a humidified staining tray with tissue sections facing up: gently wipe the area around the tissue section with paper towel and draw a closed circular line with Pap Pen to create a hydrophobic barrier to prevent a leakage of reagents from the slide during the incubation steps.
2. Cover tissue section with the blocking reagent (OOMB00003) and incubate for 20 minutes. This step is needed to block non-specific background staining.
3. Discard blocking reagent and cover tissue section with permeabilization buffer (OOMB00004) and incubate for 20 minutes. This step is needed to facilitate penetration of primary antibodies into the tissue and get a stronger specific staining.
4. Make a working dilution of primary antibodies in antibody diluent (OOMB00001). Add primary antibodies to tissue sections and incubate either overnight at 4°C or for 1-3 hours at room temperature.
5. Discard primary antibodies and wash slides 3 x 15 min with the wash buffer (OOMB00005).
6. Make a working dilution of biotinylated secondary antibodies in antibody diluent (OOMB00001). Add to tissues sections for 40-60 minutes.
7. Discard secondary antibodies and wash slides 3 x 15 min with the wash buffer.
8. Make a working dilution of Streptavidin-HRP conjugate and add to tissue sections: incubate for 30 minutes.
9. Discard Steptaivdin-HRP solution from slides and rinse slides 3 x 1 minute with wash buffer.
10. Calculate the required working volume of either DAB or AEC Chromogen Substrate Solution (250 µL is usually needed for tissue section): add to tissue section for 5-15 minutes. Appearance of color may be monitored under the bright-field microscope. DAB is carcinogen and therefore personal protection is required for handling this reagent.
11. Discard Chromogen Substrate Solutions from the slides (follow MSDS instructions to discard DAB reagent).
12. Rinse tissue sections 3 x 2 minutes with wash buffer and then rinse with distilled water.
13. If needed, counterstain tissue sections with hematoxylin.
14. Mount tissue sections using under the coverslips: AEC is soluble in xylene and alcohol and therefore for mounting slides stained with this chromogen aqueous mounting media needs to be used. DAB is a stable chromogen and tissue slides stained with it can be mounted with either xylene-based or aqueous mounting media.
15. Place coverslip slides vertically on the long side for 2-5 minutes to drain the excess of the mounting media.
16. Now stained tissues sections can be examined under the bright-field microscope.

For AP staining

1. Place slides horizontally into a humidified staining tray with tissue sections facing up: gently wipe the area around the tissue section with paper towel and draw a closed circular line with Pap Pen to create a hydrophobic barrier to prevent a leakage of reagents from the slide during the incubation steps.
2. Cover tissue section with the blocking reagent (OOMB00003) and incubate for 20 minutes. This step is needed to block non-specific background staining.
3. Discard blocking reagent and cover tissue section with permeabilization buffer (OOMB00004) and incubate for 20 minutes. This step is needed to facilitate penetration of primary antibodies into the tissue and get a stronger specific staining.
4. Make a working dilution of primary antibodies in antibody diluent (OOMB00001). Add primary antibodies to tissue sections and incubate either overnight at 4°C or for 1-3 hours at room temperature.
5. Discard primary antibodies and wash slides 3 x 15 min with the wash buffer (OOMB00005).
6. Make a working dilution of biotinylated secondary antibodies in antibody diluent (OOMB00001). Add to tissues sections for 40-60 minutes.
7. Discard secondary antibodies and wash slides 3 x 15 min with the wash buffer.
8. Make a working dilution of Streptavidin-AP conjugate and add to tissue sections: incubate for 30 minutes.
9. Discard Steptaivdin-AP solution from slides and rinse slides 3 x 1 minute with wash buffer.
10. Calculate the required working volume of BCIP-NBT Substrate Solution (250 µL is usually needed for tissue section): add to tissue section for 5-15 minutes. Appearance of color may be monitored under the bright-field microscope.
11. Discard Chromogen Substrate Solutions from the slides.
12. Rinse tissue sections 3 x 2 minutes with wash buffer and then rinse with distilled water.
13. If needed, counterstain tissue sections with hematoxylin.
14. Mount tissue sections using under the coverslips: BCIP-NBT is a stable chromogen and tissue slides stained with it can be mounted with either xylene-based or aqueous mounting media.
15. Place coverslip slides vertically on the long side for 2-5 minutes to drain the excess of the mounting media.
16. Now stained tissues sections can be examined under the bright-field microscope.

Reagents

1. Antibody Dilution Buffer for IHC, ICC (OOMB00001)
2. LightOn Anti-Fade Mounting Media for Immunofluorescence ICC & IHC (OOMB00002)
3. Blocking Buffer for Immunocytochemistry and Immunohistochemistry (OOMB00003)
4. Permeabilization Buffer for Immunocytochemistry and Immunohistochemistry (OOMB00004)
5. 20x Wash Buffer for IHC, ICC (OOMB00005)
6. 10X Antigen Retrieval Solution pH 6.0 for Immunohistochemistry (OOMB00006)
7. 10X Antigen Retrieval Solution pH 9.0 for Immunohistochemistry (OOMB00007)