Immunohistochemistry (or IHC) Protocol for frozen sections

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Abstract

Goal:

Immunohistochemistry (or IHC) is a method that allows demonstrating the presence and location of proteins in tissue sections.

Day 1:

1. Thaw the slides about 15 minutes.
2. Wash the slides 1 x 15 minutes in PBS with gentle agitation.
3. Wipe away excess liquid around the section on the glass slide with tissue paper.
4. Encircle the tissue section or draw lines on both sides of the section and let dry (10-15 seconds). The PAP PEN is designed to provide a water repellant barrier when a circle is drawn around a specimen such as tissue sections or cells. The barrier of the PAP PEN retains antisera within the defined area and ensures that only the amount of antibody needed for sufficient reaction is used.
5. Block in 5% normal serum with 2% BSA in PBS plus 0.3% Triton X-100 (block solution) for 4 hours at room temperature.
6. Drain slides for a few seconds (do not rinse)
7. Wash the slides 1 x 15 minutes in PBS with gentle agitation.
8. Wipe away excess liquid around the section on the glass slide with tissue paper.
9. Apply primary antibody diluted in block solution
10. Incubate overnight at 4°C

Day 2

1. Drain slides for a few seconds (do not rinse)
2. Wash the slides 2 x 15 minutes in PBS 0.05% Tween 20 with gentle agitation
3. Wash the slides 1 x 15 minutes in PBS with gentle agitation
4. Wipe away excess liquid around the section on the glass slide with tissue paper.
5. Apply fluorophore-conjugated secondary antibody to the slide diluted to the concentration recommended by the manufacturer in block solution, and incubate for 2 hours at room temperature. This step should be done in the dark to avoid photobleaching.
6. Wash the slides 2 x 15 minutes in PBS 0.05% Tween 20 with gentle agitation
7. Wash the slides 1 x 15 minutes in PBS with gentle agitation
8. Wipe away excess liquid around the section on the glass slide with tissue paper.
9. Apply DAPI 1:100 in PBS 1X for 5 min at room temperature.
10. Rinse in running tap distilled water
11. Coverslip with mounting medium.

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Attention!! All incubations should be carried out in a humidified chamber to avoid drying of the tissue.

Controls

To estimate the contribution of the non-specific interaction and Fc receptor binding, staining protocols using an antibody directed to an irrelevant antigen (for example, BrdU) having the same isotype as the antibody of interest may be analyzed in parallel with the antibody of interest. The antibody directed to the irrelevant antigen is known as the isotype control. For whole serum antibodies, use normal serum from an unimmunized animal of the same species as the primary antibody. If an isotype control is not available, a negative antibody control is recommended. Simply replace the primary antibody with antibody diluent. A positive tissue control is strongly recommended to ensure that the antibody is performing as expected. Depending on the experiment, it may also be useful to include a negative tissue control: a tissue in which the protein of interest is not expected to be found.

References

www.abcam.com/technical

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