

IHC5

For use with unconjugated monoclonal and polyclonal antibodies.

This method provides a general procedure for use with the majority of Bio-Rad reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Please note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications.

Reagents:**1. 0.3 H₂O₂ 70% methanol in TBS**

- 1 ml of 30% H₂O₂ per 100 ml methanol/TBS

2. TBS stock solution (10x)

- NaCl, 87.66 g
- Tris base, 60.55 g
- Distilled water, 1 liter
- Adjust pH to 7.4 using concentrated HCl

Method:

1. De-paraffinize sections thoroughly in xylene/synthetic solvent, and hydrate through a graded series of alcohols. Wash twice in water.
2. Outline section with PAP pen.
3. If required, treat with 0.3% (w/v) H₂O₂ in methanol for 15 minutes to block endogenous peroxidase activity (2% (w/v) H₂O₂/methanol can be used for a shorter time if preferred; Bio-Rad offers peroxide blocking reagent (BUF017B)). Wash 3 times in TBS.
4. If required, include an appropriate antigen retrieval step to enhance the immunostaining (see protocol: Antigen Retrieval Techniques for use with Formalin-Fixed Paraffin-Embedded Sections). Bio-Rad offers a variety of retrieval/antigen unmasking fluids. Wash once in water.
5. Incubate sections for 10 minutes in 10% normal serum from the same species in which the secondary was raised in. Tap excess serum off the slides before staining.
6. Incubate sections with primary antibody for at least 30 minutes at room temperature in a humid chamber or overnight at 4°C. Wash 3 times in TBS.
7. Add biotinylated secondary antibody at the recommended dilution (see specific datasheet for details). Incubate for at least 30 minutes at room temperature. Wash 3 times in TBS.

8. Add enzyme-conjugated streptavidin at the recommended dilution (see specific datasheet for details). Incubate for at least 30 minutes at room temperature. Wash 3 times in TBS.
9. Incubate with the appropriate substrate solution for the recommended period of time (Bio-Rad suggests the use of DAB substrate with HRP-conjugated antibodies, and Fast Red/Naphthol AS-MX for Alkaline Phosphatase-conjugated antibodies). Wash once in water.
10. Counterstain with hematoxylin for 1 minute. "Blue" with running water for 5 minutes. Then wash.
11. Mount in aqueous mounting medium, or alternatively dehydrate through a graded series of alcohols and xylene/solvent, and mount in synthetic mountant.

Notes:

- Do not allow slides to dry out after the fixation step, as drying will result in damage to the tissue structure.
- Beware, certain substrates are soluble in alcohol – please refer to supplier information for details.
- Appropriate controls should always be carried out. It may be useful to include a sample in which no primary antibody is used at all, in order to determine any non-specific binding of the secondary reagent to the target tissue.

Are you interested in learning more about which reagents to use in your immunohistochemistry experiments? Bio-Rad's immunohistochemistry application page enables you to quickly and easily find antibodies, kits and reagents tested in immunohistochemistry.

Find out more at: bio-rad-antibodies.com/ihc-resources