

Worksheet IH3

PAP/APAAP Immunostaining of Frozen Tissue Sections

For use with Biozol's unconjugated monoclonal and polyclonal antibodies.

Note:

This method provides a general procedure for use with the majority of Biozol 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.

1. Prepare slides in appropriate manner. In most cases Biozol recommends that tissues are snap-frozen in liquid nitrogen, 4-6 μ m sections prepared using a cryostat.
2. Allow sections to air dry for at least 1 hour.
3. Fix sections in cold dry acetone for 10 minutes.
4. Block endogenous peroxidase, if necessary, by immersing slides in 0.3% H₂O₂ in 70% methanol/TBS for 30 minutes. Wash once in TBS.
5. Incubate sections for 10 minutes in 10% normal serum from species in which secondary was raised. Tap excess serum off the slides before staining.
6. Incubate sections in primary antibody for at least 1 hour at room temperature in a humid chamber or overnight at 4°C. Wash three times in TBS.
7. Add bridging secondary antibody at recommended dilution (see specific datasheet for details). Incubate for at least 30 minutes at room temperature. Wash three times in TBS.
8. Add enzyme complex at recommended dilution (see specific datasheet for details). Incubate for at least 30 minutes at room temperature. Wash three times in TBS.
9. Incubate in appropriate substrate solution for recommended period of time. (Biozol recommends 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 in haematoxylin, 1-10 minutes. 'Blue' in running water for 5 minutes.
11. Mount in aqueous mounting medium e.g. Histotec (BZL01014) or alternatively dehydrate through alcohols and xylene/solvent and mount in synthetic mountant.

Notes:

- N.B.** Do not allow slides to dry out after the fixation step, as drying will result in damage to the tissue structure.

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Note that certain substrates are soluble on alcohol - Please refer to supplier information for details.

Appropriate control samples should always be included. It may be useful to include a control in which no primary antibody is used at all, to determine any non-specific binding of the secondary reagents to the target tissue.

Please contact Biozol's Technical Services Department for details of recommended secondary reagents for specific applications.

Solutions used:

30% H₂O₂, 70% methanol in TBS

Immerse slides in 0.3% H₂O₂ in 70% methanol/TBS (1 ml 30% H₂O₂ per 100 ml methanol/TBS) for 30 minutes.

TBS (stock solution x10 concentrated)

Sodium chloride 87.66 g, Tris 60.55 g, Distilled water 1 litre. Adjust pH to 7.4 using concentrated HCl.

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