

Worksheet IH6

PAP/APAAP immunostaining of paraffin embedded 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. De-paraffinise sections thoroughly in Xylene/synthetic solvent and hydrate through graded series of alcohols. Wash twice in TBS.
2. If required, treat with 0.3% (w/v) hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase activity (2% w/v H₂O₂/methanol can be used for a shorter time if preferred). Wash once in TBS.
3. If required, include appropriate antigen retrieval step to enhance immunostaining (see separate Biozol Worksheet No. IH7). Wash once in TBS.
4. Incubate sections for 10 minutes in 10% normal serum from species in which bridging antibody was raised. Tap excess serum off the slides before staining.
5. Incubate sections in primary antibody for at least 1 hour at room temperature or overnight at 4°C. Wash three times in TBS.
6. 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.
7. 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.
8. 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.
9. Counterstain in haematoxylin, 1-10 minutes. 'Blue' in running water for 5 minutes.
10. Mount in aqueous mounting medium eg. Histotec (BZL01014) or alternatively dehydrate through alcohols and xylene/solvent and mount in synthetic mountant.

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Notes:

N.B. Note that certain substrates are soluble in 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 reagent to the target tissue.

Please contact Biozol's Technical services department for details of recommended secondary reagents for specific applications.

Solutions used:

TBS (stock solution x10 concentrated)

Sodium chloride 87.66 g, Tris 60.55 g, Distilled water 1 litre. Adjust pH to 7.5 using concentrated HCl.

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