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ProductInformation

Actin, α-Smooth Muscle, Immunohistology Kit

Product Number **IMMH2**
Storage Temperature 2–8 °C

For Immunohistochemical Demonstration of α-Smooth Muscle Actin in Paraffin-embedded and Frozen Human Tissue Sections

Product Description

The introduction of immunohistochemical techniques has ushered a new era of staining into the laboratory based upon sensitive, specific methods.[1,2] Using antigen-antibody relationships, tissue components previously undetected can be precisely identified.

In the Sigma procedure, an antigen-specific primary antibody is applied to deparaffinized or frozen hydrated tissue sections. Following a brief wash, the section is incubated with a biotinylated secondary antibody. Upon the addition of an ExtrAvidin®–Peroxidase reagent, a stable avidin-biotin complex is formed with the bound biotinylated antibody. The sites of antibody deposition are visualized by the addition of freshly prepared substrate, which contains hydrogen peroxide and the chromogen 3-amino-9-ethylcarbazole (AEC; an electron donor). The bound peroxidase catalyzes the oxidation of the AEC to form a reddish-brown insoluble precipitate at the antigen sites. Compared to the classic PAP procedures, avidin-biotin techniques are particularly valuable as background staining is virtually eliminated while the specific reaction is amplified.[3,4]

Actin (43 kDa) is a structural and contractile microfilament protein showing extensive interspecies and intertissue conservation. The primary antibody contained in this kit specifically identifies the alpha isotype of smooth muscle actin and reacts with normal and neoplastic smooth muscle and myoepithelieal cells.[5]

Components

Primary Antibody, Vial 1 – Mouse Monoclonal anti-α-Smooth Muscle Actin in buffered saline. Sodium azide, 0.1%, added as preservative.

Biotinylated Secondary Antibody, Vial 2 – Goat anti-Mouse Immunoglobulin in buffered saline. Sodium azide, 0.1%, added as preservative.

Peroxidase Reagent, Vial 3 – ExtrAvidin-conjugated Peroxidase in buffered saline. Preservative added.

Acetate Buffer, Vial 4a – Acetate buffer, 2.5 mol/L, pH 5.0.

AEC Chromogen, Vial 4b – 3-amino-9-ethylcarbazole (AEC) in N,N-dimethylformamide.

Hydrogen Peroxide, Vial 5 – 3% H_2O_2 in deionized water.

Mixing Vial, Vial 6

Reagents and Equipment Required by not Provided

Blocking Reagent: normal goat serum (Product No. G9023), 1% (v/v) in buffered saline

Negative Control: normal mouse serum (Product No. M5905), 5% (v/v) in buffered saline

Phosphate buffered saline, pH 7.4 (available as tablets, Product No. P4417)

Deionized water

Mayer's Hematoxylin Solution (Product No. MHS1)

Glycerol Gelatin (Product No. GG1)

Slides, coverslips

Humidity chamber (Product No. H6644)

Light microscope

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Primary Antibody and Biotinylated Secondary Antibody contain sodium azide. Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

AEC chromogen contains 3-amino-9-ethylcarbazole. AEC is harmful if swallowed, inhaled, or absorbed through skin. AEC is a possible carcinogen. Avoid all contact. Wear protective clothing. Wash thoroughly after handling.

Storage/Stability
Store at 2–8 °C

Procedure
Specimen Separation
Tissues fixed in 10% neutral buffered formalin, B-5 fixative, or Bouin's solution[12] can be used. Cut tissue sections at 4-6 microns. Acetone fixed frozen sections may also be used. For detection of antigens requiring unmasking, digest formalin fixed material with 0.1% trypsin (Product No. T7409) or 0.1% protease (Product No. P5147) prior to the first step in the procedure.

Note: Since tissue sections have a tendency to fall off during immunohistochemical procedures, Poly-L-Lysine (Product No. P8920) may be used as a tissue adhesive.

Controls
For the correct interpretation of the staining results it is necessary to run a positive control tissue section known to contain the antigen in question and a negative control test section incubated with the negative control reagent.

All rinses are with phosphate buffered saline (PBS), pH 7.4. Following incubations slides should be washed gently with PBS from a wash bottle, avoid a direct jet of liquid, which may wash off or loosen sections. Carefully wipe each slide free of excess fluid before the application of the next reagent. Avoid touching the tissue section. Be certain to apply enough drops of the reagent to cover the sections.

DO NOT allow the tissue sections to dry out at any time during the procedure. It is recommended the incubations be performed in a humidity chamber. All incubations are at room temperature unless otherwise specified. Wash steps can include placing slides in a PBS bath for 2 minutes.

1. Deparaffinize and hydrate sections to water.
2. Quench endogenous peroxide with 2 drops of 3% hydrogen peroxide (Vial 5) for 5 minutes. Wash and wipe slides.
3. Incubate with Blocking Reagent for 10 minutes. Wipe off excess reagent, but do not wash slides.
4. Apply 2 drops of Primary Antibody (Vial 1) or Negative Control and incubate 60 minutes. Wash and wipe slides.
5. Apply 2 drops of Biotinylated Secondary Antibody (Vial 2) and incubate 20 minutes. Wash and wipe slides.
6. Apply 2 drops of Peroxidase Reagent (Vial 3) and incubate 20 minutes. Wash and wipe slides.
7. Prepare Substrate Reagent in Mixing Vial (Vial 6)
   In order add:
   4 ml of deionized water
   2 drops of Acetate Buffer (Vial 4A)
   1 drop of AEC Chromogen (Vial 4B)
   1 drop of 3% hydrogen peroxide (Vial 5)
8. Apply 2 drops of Substrate Reagent and incubate up to 10 minutes. Check slide microscopically for adequate chromogen development.
9. When sufficient staining has been achieved rinse slides in deionized water for 5 minutes. Wipe off excess.
10. Counterstain with Mayer's Hematoxylin for 2 minutes.
11. Rinse in gently running tap water to "blue" the hematoxylin.
12. Apply glycerol gelatin or other aqueous mounting media and carefully cover with coverslip.

Results
Nuclei will be blue, while the cytoplasm of positive cells will be rose-red to brownish-red.

References

1. Taylor, C.R., Immunoperoxidase Techniques. Practical and Theoretical Aspects. Arch. Pathol. Lab. Med., **102**, 113 (1978).
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3. Naritoku, W.Y., and Taylor, C.R., A comparative study of the use of monoclonal antibodies using three different immunohistochemical methods. An evaluation of monoclonal and polyclonal antibodies against human prostatic acid phosphatase. J. Histochem. Cytochem., **30**, 253 (1982).
4. Hsu, S.M., *et al*., Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J. Histochem. Cytochem., **29**, 577 (1981).
5. Bussolati, G., *et al*., Immunohistochemistry of Actin in normal and neoplastic tissues, in: Advances in Immunochemistry, DeLellis, R.A., ed., Masson Publishing USA, Inc., p. 325 (1984).
6. Culling, C.F., *et al*., The effect of various fixatives and trypsin digestion upon the staining of routine paraffin embedded sections by the peroxidase-anti-peroxidase and immunofluorescent technique. J. Histotechnology, **3**, 10 (1980).

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