SIGMA-ALDRICH

DESMIN

For Immunohistochemical Demonstration of Desmin in Paraffin-embedded and Frozen Human Tissue Sections
Stock No. IMMH-5

TABLE OF CONTENTS

BACKGROUND AND PRINCIPLE

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 biotinylated primary antibody is applied to deparaffinized or frozen hydrated tissue sections. Following a brief incubation and wash, the section is then incubated with the 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-ethyl-cabazole (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]

Desmin is an intermediate filament protein expressed in skeletal, cardiac and smooth muscle cells.[5] It is also present in benign and malignant myogenic tumors such as leiomyoma, leiomyosarcoma, rhabdmyosarcoma and alveolar soft part tumor. Certain types of vascular smooth muscle, early embryonal muscle and poorly differentiated neoplastic muscle cells may lack immunohistochemically detectable desmin.[6]

FOR RESEARCH USE ONLY;
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Storage: Store at 2-8 °C

REAGENTS AND EQUIPMENT PROVIDED

Biotinylated Primary Antibody, B 8278: Mouse Monoclonal anti-Desmin in buffered saline. Sodium azide, 0.1%, added as preservative.

Biotinylated Negative Control, B 8403: IgG Fraction of Normal Mouse Serum in buffered saline. Sodium azide, 0.1% added as preservative.

Peroxidase Reagent, E 1267: ExtrAvidin®-conjugated Peroxidase in buffered saline. Preservative added.

Acetate Buffer, A 0432: Acetate buffer, 2.5 mol/L, pH 5.0.

AEC Chromogen, A 0557: 3-amino-9-ethylcarbazole (AEC) in N,N-dimethylformamide.

Hydrogen Peroxide, H 0896: 3% H_2O_2 in deionized water.

Mixing Vial

PRECAUTIONS

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.

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Blocking Reagent: normal mouse serum (Sigma Product No. M 5905), 1% (v/v) in buffered saline

Phosphate buffered saline, pH 7.4 (available as tablets, Sigma Product No. P 4417)

Deionized water

Mayer's Hematoxylin Solution (Sigma Product No. MHS-1)

Glycerol Gelatin (Sigma Product No. GG-1)

Slides, coverslips

Humidity chamber (Sigma Product No. H 6644)

Light microscope

SPECIMEN PREPARATION

Tissues fixed in 10% neutral buffered formalin, B-5 fixative or Bouin's solution[1,2,7] can be used. Cut tissue sections at 4–6 microns. Accetone fixed frozen sections may also be used. For detection of antigens requiring unmasking, digest formalin fixed material with 0.1% trypsin (Sigma Product No. T 8128) or 0.1% protease (Sigma Product No. P 5147) prior to the first step in the procedure.

Note: Since tissue sections have a tendency to fall off during immunohistochemical procedures, Poly-L-Lysine (Sigma Product No. P 8920) 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.

Note: 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 water 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.

PROCEDURE

1. Deparaffinize and hydrate sections to water.
2. Quench endogenous peroxide with 2 drops 3% hydrogen peroxide (H 0896) for 10 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 Biotinylated Primary Antibody (B 8278) or Biotinylated Negative Control (B 8403) and incubate 60 minutes. Wash and wipe slides.
5. Apply 2 drops Peroxidase Reagent (E 1267) and incubate 30 minutes. Wash and wipe slides.
6. Prepare Substrate Reagent in Mixing Vial.

   In order add:

   4 ml deionized water

   2 drops Acetate Buffer (A 0432)

   1 drop AEC Chromogen (A 0557)

   1 drop 3% hydrogen peroxide (H 0896)
7. Apply 2 drops Substrate Reagent incubate up to 10 minutes. Check slide microscopically for adequate chromogen development.

PROCEDURE CONT'D

8. When sufficient staining has been achieved rinse slides in deionized water for 5 minutes. Wipe off excess.
9. Counterstain with Mayer's Hematoxylin for 2 minutes.
10. Rinse in gently running tap water to "blue" the hematoxylin.
11. Apply glycerol gelatin or other aqueous mounting media and carefully cover with coverslip.

Note: Microwave applications available upon request.

EXPECTED OBSERVATIONS

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).
2. Guesdon, J.L., et. al., The use of avidin-biotin interaction in immunoenzymatic techniques, *J. Histochem. Cytochem.*, **27**, 1131 (1979).
3. Naritoku, W.Y., and C.R. Taylor, 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 immunoper oxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures, *J. Histochem. Cytochem.*, **29**, 577 (1981).
5. Lazarides, E., Intermediate Filaments: A chemically heterogenous developmentally regulated class of proteins., *Annu. Rev. Biochem*., **51**, 219 (1982).
6. Denk, H., et al., Proteins of intermediate filaments-an immunohistochemical and biochemical approach to the classification of soft tissue tumors., *A.M. J. Pathol.*, **110**, 193 (1983).
7. 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).

SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 • (314) 771-5765
Fax (314) 286-7828

Sigma brand products are sold through Sigma-Aldrich, Inc.
Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.

Printed in the USA

kaa–10/04