α-SARCOMERIC ACTIN

Stock No. IMMH-1

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

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 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-ethyl-carbazole (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 is a 43,000 MW structural and contractile microfilament protein showing extensive inter- species and intertissue conservation. Of the six isoforms described for actin, the α-skeletal and the α-cardiac predominate, respectively in skeletal and cardiac muscle.

Detection of α-sarcomeric actin, in conjunction with desmin, myosin, myoglobin, and laminin, is useful in differentiating rhabdomyosarcomas, mesenchymal neoplasms, leiomyo- sarcomas, other myogenic sarcomas and pleomorphic neoplasms. No cross-reactivity with normal smooth muscle or non-muscle tissues has been demonstrated for antibodies to the α-isoform[5-11].

FOR RESEARCH USE ONLY;
NOT FOR USE IN DIAGNOSTIC PROCEDURES
Storage: Store at 0-5°C

REAGENTS AND EQUIPMENT PROVIDED

Primary Antibody, Vial 1: Mouse Monoclonal anti-α-Sarcomeric 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

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 goat serum (Sigma Product No. G-9023), 1% (v/v) in buffered saline
- Negative Control: normal mouse serum (Sigma Product No. M-5905), 5% (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[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 (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 immuno-histochemical 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 includeplacing slides in a PBS bath for 2 minutes.

PROCEDURE

1. Deparaffinize and hydrate sections to water.
2. Quench endogenous peroxidase with 2 drops 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 Primary Antibody (Vial 1) or Negative Control and incubate 60 minutes. Wash and wipe slides.
5. Apply 2 drops Biotinylated Secondary Antibody (Vial 2) and incubate 20 minutes. Wash and wipe slides.
6. Apply 2 drops 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 deionized water
   2 drops Acetate Buffer (Vial 4A)
   1 drop AEC Chromogen (Vial 4B)
   1 drop 3% hydrogen peroxide (Vial 5)

8. Apply 2 drops Substrate Reagent 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.

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.

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