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MOUSE ANTI-SMOOTH MUSCLE MYOSIN, HEAVY CHAIN MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB3572

LOT NUMBER:

QUANTITY: 100 µg

CONCENTRATION: 1 mg/mL

SPECIFICITY: Smooth muscle myosin, heavy chain. By Western blot the antibody recognizes a protein of 200-204 kDa.

IMMUNOGEN: Crude smooth muscle extract from normal human adult uterus.

ISOTYPE: IgG_1

CLONE: V/10 (also referred to as SM-M10)

APPLICATIONS: **Western blot** (see application notes on back). Suggested blocking buffer is TBS-Tween with 2% BSA. Suggested dilution buffer is TBS-Tween with 0.05% sodium azide. Preferred gel percentage is 5% (see application notes).

Immunohistochemistry on frozen and paraffin embedded tissue sections. Suggested fixation for frozen tissue sections is acetone fix for 6 minutes at room temperature. For formalin fixed paraffin embedded tissue sections: microwave in 0.01M citrate buffer (pH 6.0) for 8-10 minutes (note that all microwaves differ and adjustments may need to be made) follow with enzyme digestion (0.01% pronase for 10 mintues). Suggested blocking agent is fetal bovine serum. The antibody has also been used successfully on methyl-Carnoy fixed tissue.

Immunocytochemistry

Immunoprecipitation. Suggested extraction buffer is 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% deoxycholic acid-NaCl and 0.5 mM PMSF. Final reaction volume is 1 mL and suggested capture agent is agarose conjugated anti-mouse IgG.

Optimal working dilutions must be determined by the end user.

POSITIVE CONTROL: Smooth muscle (e.g. aterial tunica media). Negative control: any nonmuscle tissue (e.g. arterial tunica adentitial).

SPECIES REACTIVITIES: Human, bovine, porcine and rabbit. Other species have not been tested.

FORMAT: Purified immunoglobulin.

PRESENTATION: Liquid in 0.02M Phosphate buffer with 0.25M NaCl and 0.1% sodium azide.

STORAGE/HANDLING: Maintain at 2-8°C in undiluted aliquots up to 6 months after date of receipt

REFERENCE: Frid et al. Multiple phenotypically distinct smooth muscle cell populations exist in the adult and developing bovine bovine pulmonary arterial media in vivo. Circ Res 1994;75:669-681.

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APPLICATION NOTES FOR MAB3572

WESTERN BLOT

To achieve good resolution of myosin heavy chain isoforms with distinct molecular weight (200 – 2004 kDa), the following procedure should be followed: 1). Pyrophoshate extraction buffer for sample preparation (see below); run SDS-PAGE in 5% gel. Important: for better resolution of the MHC bands, use electrophoretic buffer with pH 8.2 (i.e. 0.1 less than standard), and prepare resolving gel (5%) with pH 9.0 (not 8.8 as usual). Also help thorough degasing of the resolving gel mixture (H2O, acrylamide, EDTA, pH 9.0, before (!) adding SDS, TEMED and APS). Run SDS-PAGE longer than after the dye front runs off (use 200 kDa MW markers and let it's 200 kDa band run at least to the middle of 8X8 gel (using a big size gel (not the mini-gel!) will enhance the quality of MHC band resolution).

Pyrophosphate extraction buffer: (40mM $Na_4P_2O_7x10H_2O$, 1mM $MgCl_2$, 1mM EGTA (add KOH to dissolve EGTA), PMSF, pH 9.5). To extract acto-myosin from tissues/cells, shake minced tissue or cells in cold extraction buffer 1 hr on ice bath (0^oC), centrifuge @10,000g for 10 min at +2-8 ^oC, take supernatant and mix it 1:1 with standard Laemmli sample buffer, boil, run SDS-PAGE in 5% gel (see above).

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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