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MOUSE ANTI-VINCULIN MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB3574

LOT NUMBER:

QUANTITY: 100 µg

CONCENTRATION: 1 mg/mL

SPECIFICITY: Vinculin. By Western blot the antibody recognizes a protein of 130-kDa.

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

ISOTYPE: IgG_1

CLONE: 7F9

APPLICATIONS:

Western blot. Suggested blocking buffer is TBS-Tween with 2% BSA. Suggested dilution buffer is TBS-Tween with 0.05% sodium azide. Preferred gel percentage is 10% and/or 4-20% (or similar) gradient gel.

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). If necessary 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 (see application notes on back).

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. Note that the muscle-specific isoform, meta-vinculin (MW 150 kDa) will co-precipitate.

Optimal working dilutions must be determined by the end user.

POSITIVE CONTROL: Vinculin is present in all cell types.

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

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REFERENCES:

1). Glukhova MA, Frid MG, Koteliansky VE: "Developmental changes in expression of contractile and cytoskeletal proteins in human aortic smooth muscle". J Biol Chem 1990; 265:13042-13046
2). Frid MG, Moiseeva EP, Stenmark KR: "Multiple phenotypically distinct smooth muscle cell populations exist in the adult and developing bovine pulmonary arterial media in vivo". Circ Res 1994;75:669-681.
3) Lemler MS, Bies RD, Frid MG, et al.: "Myocyte cytoskeletal disorganization and right heart failure in hypoxia-induced neonatal pulmonary hypertension". Am J Physiol Heart Circ Physiol 2000; 279: H1365-H1376.

RELATED REFERENCES: 1) Belkin AM et al: "Diversity of vinculin/meta-vinculin in human tissues and cultured cells". J Biol Chem 1988, 263:6631-6635.

2) Belkin AM et al.: "Immunolocalization of meta-vinculin in human smooth and cardiac muscles". JCB 1988; 107:545-553.

APPLICATION NOTES FOR MAB3574

IMMUNOCYTOCHEMISTRY

For cultured cells, use the following method: 1) wash cells twice with buffer "A" (100 mM Pipes (to make it dissolve, titrate with KOH), 0.5mM MgCl2, 0.1 mM EDTA, 0.01M EGTA (to make it dissolve, titrate with KOH), pH 6.9); 2) fix 15 min @RT (3.7% formalin, 0.2% Triton X-100, 2M Glycerol, prepared on buffer A); 3) wash 3 times with buffer "A"; 4) proceed with incubation with primary Abs, etc.

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.*

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