Biomedical Technologies Inc.

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DATA SHEET

anti-Smooth Muscle Myosin IgG

Catalog No: BT-562

Lot No: 5620305

Quantity: 0.5ml

Packaging: BTI rabbit anti-Myosin IgG (smooth muscle) is packaged in 0.5ml aliquots with 1mM sodium azide added as preservative.

Storage & Stability: Stable for one year at -80◦C. Refrigerated at 4◦C it is stable for 6 months. Avoid repeated freeze thawing.

Preparation: Rabbits were immunized with bovine tracheal smooth muscle myosin 98% Pure via SDS-PAGE. The IgG was purified from immune serum via ammonium sulfate precipitation followed by ion exchange chromatography. The IgG was dissolved, dialyzed and packaged in 150mM NaCl, 1mM sodium azide, 20mM Tris, pH 7.5.

Specificity: BTI anti-Myosin IgG (smooth muscle) will bind only smooth muscle myosin II heavy chain in vertebrates. It contains no detectable cross reactivity with any other protein including the following based on Western Blot analysis:

Myosin - Skeletal
Myosin - Non Muscle

Whole cell extracts (in ice) should be boiled in SDS sample buffer. Apply 0.1-2ug Protein to each lane of SDS acrylamide gels, electrophorese and transfer to nitrocellulose, incubate overnight at 10◦C with this antibody (1:150) in Tris buffered Saline with 3% horse serum and then envisioned with GAR IgG HRP.

Usage: This antibody can be used to specifically stain cultured smooth muscle cells and to identify vascular and other types of smooth muscle in tissue sections.

References: 1. J. Kolega. J Cell Science 111:2085-2095 (1998).
2. F. Cornacchia, et al. J. Clin. Invest. 108: 1649-1656 (2001).
3. B. Hinz, et al. Am. J. of Pathology 159:1009-1020 (2001).

PROCEDURE FOR IMMUOFLUORESCENCE WITH
anti-Myosin IgG (Smooth-Muscle)

1. Rinse cells with phosphate buffered saline (PBS). Use 100ul for each coverslip and submerge cells attached to the coverslips with the cells facing up. Wait one minute.

2. Fix cells at ambient temperatures (37◦C) for 5-10 minutes in freshly prepared formaldehyde (from paraformaldehyde powder) at a concentration of 2-4% buffered with PBS.

3. Rinse cells in PBS. 2-3 changes, 5-10 minutes per wash.

4. Incubate cells in 0.1% Triton X-100, 0.1% Deoxycholate in PBS for 7 minutes at room temperature.

5. Without allowing the cells to air dry, repeat step 3.

6. Prepare antibody solutions as follows:

   a. Clarify by centrifugation in Eppendorf Microfuge 1-2 minutes at 4◦C.
   b. Pipet from the supernatant without disturbing the pellet of aggregated IgG's.
   c. Deliver 1ul antibody into 300ul of PBS containing 3% BSA, without vortexing, mix well. This is a 1:300 dilution. 300ul of this dilution is sufficient to stain 5 to 7 coverslips (18mm).

7. Incubate cells with antibodies for 90-120 minutes at room temperature by inversion of coverslips with cells onto a drop of antibodies in a moist chamber to prevent evaporation.

8. Rinse cells with PBS containing 3% BSA, 3-4 changes, 5-10 minutes per wash.

9. Incubate cells with appropriate dilution (dilute with PBS containing 3% BSA) of labeled secondary antibody as in steps 6,7.

10. Repeat step 8.

11. Rinse cells in PBS. 3-5 changes, 5-10 minutes per wash.

12. Mount coverslips onto cleaned microscope slides in a 20ul drop of mounting media or glycerin and BSS (9:1). Seal slips at the edges with nail varnish. Excess mounting media can be conveniently removed with laboratory tissue by capillary prior to sealing.

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