Biomedical Technologies Inc.

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DATA SHEET

anti-Actin IgG

Catalog No: BT-560

Quantity: 0.5ml [OD (280) 1:50 dilution = 0.2 = 0.14 mg/ml] or [3.5mg Protein/0.5ml]

Lot No: 5600904

Packaging: BTI anti-Actin IgG (rabbit) is packaged in 0.5ml aliquots with 0.02% Sodium Azide added as preservative.

Storage & Stability: Stable for one year at -80◦C. Refrigerated at 4◦C it is stable for six months.

Actin immunogen run on a 7.5% acrylamide gel with 0.1% SDS stained with coomassie blue.

Preparation: Rabbits were immunized with homogenous Actin (see gel) purified from Chicken gizzards. The IgG was purified from immune serum by repeated precipitations with ammonium sulfate. The IgG was dissolved, dialyzed and packaged in phosphate buffered saline.

Specificity: BTI anti-Actin IgG will bind F and G Actins from both muscle and non-muscle tissues from all vertebrate and invertebrate species. BTI anti-Actin IgG contains no detectable cross-reactivity with any other protein including the following:

Tubulin
Keratin
Myosin

Protein A [I-125] labeled BTI anti-Actin IgG binds only Actin in whole cell homogenates electrophoresed in SDS acrylamide gels.

***Special Note on Immunoblotting muscle/non-muscle samples**. Protein A [I-125] labeled BTI anti-Actin IgG binds only Actin in whole cell homogenates electrophoresed in SDS acrylamide gels.

Skeletal Muscle: Immunoblotting Actin from skeletal muscle should be avoided due to the low affinity of antibody for skeletal muscle Actin. However, skeletal muscle Actin can be envisioned immunohistochemically with BTI anti-Actin IgG.

FOR RESEARCH USE ONLY

PROCEDURE FOR IMMUNOFLUORESCENCE

1. Rinse cells with a balanced salt solution (BSS). Use 100ml 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 2-3% buffered with BSS.

3. Rinse cells in BSS. 2-3 changes, five-ten minutes per wash with gentle agitation.

4. Dehydrate cells with absolute acetone cooled to dry ice temperatures for 1-3 minutes by plunging coverslips into a 100ml beaker filled with solvent.

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 pellet of aggregated IgGs.
   c. Make appropriate dilution (undiluted to 1:30). **Example:** Deliver 4ul of antibody into 96ul BSS without vortexing, mix well. This is a 1:25 dilution. 100ul of this dilution is sufficient to stain 1 or 2 coverslips (18mm).

7. Incubate cells with antibodies for 60-90 minutes at room temperature by inversion of coverslips with cells onto a drop of antibodies in a chamber to prevent evaporation.

8. Repeat step 3.

9. Incubate cells with appropriate dilution of labeled secondary antibody as in steps 6 and 7.

10. Repeat step 3.

11. Mount coverslips onto cleaned microscope slides in a 25ul 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 capillarity prior to sealing.

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