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Purified Mouse Anti-Actin Monoclonal Antibody

CLT9001
LOT: 2895655

DESCRIPTION:

Actin is an abundant cytoskeletal protein found in all cells [(1)]. The protein's 42 kDa peptide chain assumes two physical forms: globular actin (G-actin), which may serve as a cytoplasmic storage pool, and fibrous actin (F-actin) which, in conjunction with myosin, generates muscle contraction [(1)].
In non-muscle cells, actin appears to be involved in a variety of functions such as cell motility, exocytosis and phagocytosis [(1)]. The distribution of the six known isoforms of actin (four muscle actins: α-skeletal, α-vascular smooth, α-cardiac, and γ-enteric smooth and two cytoplasmic actins: γ and α) is tissue specific [(2,3)].

This is a pan-actin antibody that binds to an epitope in a highly conserved region of actin, therefore it reacts with all six isoforms of vertebrate actin [(3)]. The recognized epitope appears to be located in the N-terminal two thirds of the actin molecule, possibly near amino acids 50-70. Both globular (G) and filamentous (F) actin forms are recognized. In addition to labeling myotubules, anti-actin stains myoblasts and fibroblasts [(4)]. Although clone C4 is prepared as an antibody to chicken gizzard muscle actin, it reacts with actins from all vertebrates, as well as with *Dictyostelium discoideum* and *Physarum polycephalum* actins [(3)].

PRESENTATION:

100 µg liquid in 0.1 M Tris-Glycine (pH 7.4), 150 mM NaCl with 0.05% sodium azide (NaN_3). This antibody was purified from ascites fluid by protein G fractionation.

STORAGE/STABILITY:

Maintain at 4°C in undiluted aliquots for up to 1 year after date of receipt.

SPECIFICATIONS:

Clone: C4

Immunogen: Chicken Gizzard actin

Ig Class: Mouse IgG2b

Concentration: 1 mg/ml

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In CANADA: Toll Free: 1-800-268-5058
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Recommended Working Concentration:

10 µg/ml - Immunocytochemistry (100 tests)
10 µg/ml for paraffin embedded, 4% formaldehyde, 3% gluteraldehyde, sodium cacodylate treated sections [11].
10 - 50 µg antibody/ml - Western Blots (colorimetric)
4 µg/ml - Western Blots (chemiluminescent)
0.135 µg/ml - ELISA

ANALYSIS:

Immunocytochemistry:

Immunocytochemistry is performed on chamber slide cultures of 3T3 mouse fibroblasts according to the procedure described in the "Applications" section below. Anti-actin stains cytoplasmic actin in the 3T3 cells.

Titer (ELISA):

The titer of each lot of anti-actin is determined by an ELISA (enzyme-linked immunosorbent assay). Microtitration plate wells that are coated with 10 µg/ml of bovine actin are blocked with 1% bovine serum albumin (in phosphate bufered saline [PBS]) for 30 min. at room temperature, washed with PBS, and then incubated with 0-100 µg/ml anti-actin for 1 hour at room temperature. Afterwards, the wells are washed with PBS and incubated with a peroxidase-conjugated rabbit anti-mouse Ig for 1 hour at room temperature. After another PBS wash, the wells are reacted with ABTS, and the plates are read at 405 nm. Titer is defined as the concentration that gives half the maximal optical density reading.

RECOMMENDED PROCEDURES:

These procedures represent suggested guidelines for the use of anti-actin. Antibody concentrations and incubation conditions for a given experimental system should be determined empirically.

1. IMMUNOCYTOCHEMISTRY

The procedure below was developed to localize actin in cultured 3T3 mouse fibroblast cells. Cells are cultured on chamber slides or coverslips. Perform all steps at room temperature, unless otherwise indicated.

Fixing cultures

1. Fix cultures in cold methanol for 7 min at -20°C,
2. Wash three times with fresh PBS, 5 min per wash.

Application of anti-actin

1. To block non-specific staining, incubate cultures for 60 min in PBS containing normal animal serum, 1% (v/v) and BSA 0.1% (w/v). (The serum should be from the same species as the secondary antibody).
2. Gently dispense anti-actin onto the surface of the cells. An antibody concentration of 10 µg/ml works well for most applications. Working dilutions of anti-actin should be freshly prepared on the day of use. To be certain to cover the surface of all cells, use approximately 100 µl/coverslip or 500 µl/chamber.
3. Incubate in a humid chamber for 60 min.
4. Wash three times with PBS, 5 min per wash.

Detection with secondary antibody

1. Gently dispense an appropriate amount of fluorescein-conjugate (FITC) anti-mouse IgG $F(ab')_2$ diluted 1:200 in PBS containing normal animal serum, 1% (v/v) and BSA 0.1% (w/v) onto the surface of the cells, using the same concentration as was used with the primary antibody.
2. Incubate for 60 min.
3. Wash three times with PBS, 5 min per wash. (Peroxidase-conjugated anti-mouse IgG $F(ab')_2$ may also be used.)

   Other secondary antibody detection systems may be used [5-7]. For optimal results, both the primary and secondary antibody reagents should be titrated [8].

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Mount and apply coverslip

1a. For chamber slides: carefully remove the plastic chamber section. Place a small drop of aqueous mounting medium on each chamber, and place the coverslip onto the drops of media.

1b. For coverslip cultures: place a small drop of aqueous mounting media on a glass slide. Invert the coverslip and carefully lower onto the drop of mounting media.

2. WESTERN BLOT ANALYSIS

The ability of clone C4 anti-actin to recognize all isoforms of actin makes it useful as a control in Western blot analysis [2]. A standard protocol for anti-actin is summarized below.

Gel Electrophoresis

1. Dilute actin (e.g. bovine muscle) in sample buffer and load onto a SDS gradient gel alongside molecular weight markers (14.3-200kD).
2. After separation by electrophoresis, transfer the protein onto nitrocellulose.

Detection

1. Block the nitrocellulose with dry milk (5% (w/v), in H_2O) and then incubate with 10-50 μg/ml anti-actin, for 1 hour.
2. Wash the nitrocellulose, 5 minutes per wash, in PBS containing 0.01% Triton X (v/v) and twice more in PBS.
3. Incubate the nitrocellulose with alkaline phosphatase-conjugated anti-mouse IgG for 30 min on a shaker platform.
4. Visualize the complex with a BCIP/NBT colour detection system.

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