Product Information

Anti-c-Myc Antibody, Mouse Monoclonal

Clone 9E10, purified from hybridoma cell culture

M4439

Product Description

The human *c-myc* proto-oncogene is the human cellular homologue of the avian *v-myc* gene that is present in several leukemogenic retroviruses.[1,2] Increased *c-myc* expression has been described in various human tumors that occur by mechanisms such as gene amplification and chromosomal translocation.[3] The gene encodes a polypeptide with a predicted molecular weight of 49 kDa, which shows aberrant electrophoretic mobility on polyacrylamide gel electrophoresis to give an apparent molecular weight of around 62 kDa (p62c-myc).[4] p62c-myc is associated mainly with the cell nucleus, where it exerts its normal and oncogenic functions.[1,3]

An epitope located within amino acids 410-419, which contains the sequence EQKLISEEDL of human c-Myc, has been widely used as a tag in many expression vectors, to enable the expression of proteins as c-Myc-tagged fusion proteins.[5] Epitope tags provide a method to localize gene products in a variety of cell types, to study the topology of proteins and protein complexes, and to identify associated proteins. These tags also allow characterization of newly identified, low abundance or poorly immunogenic proteins when protein-specific antibodies are not available.[5,6]

Monoclonal Anti-c-Myc (mouse IgG1 isotype) is derived from the 9E10 hybridoma, which is produced by fusion of mouse myeloma cells and splenocytes from BALB/c mice. A synthetic peptide corresponding to residues 408-439 of the human p62c-myc protein conjugated to KLH was used as immunogen.[1] The isotype was determined using the IsoStrip™ Mouse Monoclonal Antibody Isotyping Kit (Cat. No. 11493027001).

Anti-c-Myc recognizes an epitope located within the sequence EQKLISEEDL (residues 410-419) of the product of the human oncogene c-*myc*.[7] The antibody recognizes the c-Myc tag sequence when it is expressed at either the amino or the carboxyl terminus of the fusion protein. The antibody reacts specifically with c-Myc-tagged fusion proteins in immunoblotting, immunoprecipitation and immunofluorescence applications. Reaction of the antibody in immunoblotting is inhibited by the c-Myc peptide (Cat. No. M2435).[6]

The antibody reacts with both components of the p62c-myc-p64c-myc doublet, by immunoblotting.[1,7] The antibody is also useful in immunohistochemical labeling of the c-Myc oncoprotein in formalin-fixed paraffin-embedded tissue sections, applying light microscopy[8] and electron microscopy.[4,8] Additional applications of the product include ELISA.[1] The antibody cross-reacts with human p62/64$^{c\text{-}myc}$, but fails to recognize the chicken p11$^{gag\text{-}myc}$ protein present in MC29 virus-transfected quail fibroblasts, nor does it react with the mouse p64/66$^{c\text{-}myc}$ protein.[1] Nevertheless, weak reaction with murine c-myc may be seen when the antibody is used at high concentration.

Several theses[9-14] and dissertations[15-34] cite use of this M4439 product in their research protocols.

Reagent

Monoclonal Anti-c-Myc is supplied as purified IgG in 0.01 M phosphate buffered saline (pH 7.4), containing 15 mM sodium azide as a preservative.

Antibody concentration: ~ 2 mg/mL (exact information on lot-specific Certificate of Analysis).

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in a frost-free freezer is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazardous and safe handling practices.

Product Profile

A minimum working dilution of 1:5000 is determined by immunoblotting of an *E. coli* extract expressing a recombinant c-Myc-tagged fusion protein. The tagged protein was detected using a chemiluminescent substrate.

Note: To obtain optimal results using different techniques and preparations, we recommend determining the optimal working dilution by titration.

Immunoprecitation

1-2 µg of the antibody can immunoprecipitate a c-Myc fusion protein from transfected mammalian cell lysates or bacterial extracts.

Note: To obtain best results and assay sensitivity in different techniques and preparations, we recommend determining optimal working dilutions by titration.

Procedure

Procedure for Immunoblotting

Note: All incubation steps should be performed at room temperature.

1. Separate c-Myc-tagged proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5-20 µg of total lysate protein per lane. The amount of lysate to be loaded per lane depends on the level of protein expression. Thus, the optimum loading may vary between preparations.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk in phosphate buffered saline (PBS, Cat. No. D8537) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween® 20 (PBS-T, Cat. No. P3563).
5. Incubate the membrane with Monoclonal Anti-c-Myc as the primary antibody for 60-120 minutes, using an optimized concentration in PBS-T.
6. Wash the membrane three times for 5 minutes each in PBS-T.
7. Incubate the membrane with anti-mouse IgG Peroxidase conjugate (Cat. Nos. A9917, A3682, or A2304) or with anti-mouse Fab Alkaline Phosphatase conjugate (Cat. Nos. A1293, A2179 or A1682) as the secondary antibody, at the recommended concentration in PBS-T, for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS-T.
9. Treat the membrane with a peroxidase or an alkaline-phosphatase substrate.

Procedure for Indirect Immunofluorescent Staining of Cultured Cells

Note: All incubation steps should be performed at room temperature, except Steps 1 and 3.

1. Grow transfected cultured cells which express a c-Myc-tagged protein on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS.
3. Fix the cells:
   a. First with -20 °C methanol (10 minutes),
   b. Then with -20 °C acetone (1 minute).
4. Wash coverslips twice in PBS (5 minutes each wash).
5. Incubate the coverslips cell-side-up with Monoclonal Anti-c-Myc in PBS containing 1% BSA, for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate the coverslips, cell-side-up, with anti-mouse Fab, FITC conjugate (Cat. No. F4018 or F8771) as the secondary antibody, at the recommended dilution, in PBS containing 1% BSA, for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
10. Examine using a fluorescence microscope with appropriate filters.

Note: Blocking with PBS containing 1% BSA for 10 minutes at room temperature, followed by draining prior to Step 5, may minimize non-specific adsorption of the antibody.

Procedure for Immunoprecipitation

1. Centrifuge 40 µL of Protein G-Sepharose® beads, 1:1 suspension (Cat. No. P3296) for 1 minute at 12,000 × g. Then wash twice with 1 mL RIPA buffer (50 mM Tris base, pH 7.4, containing 0.25% (w/v) deoxycholate, 1% Igepal® CA-630, 150 mM NaCl, and 1 mM EDTA) at 4 °C.
2. Add Monoclonal Anti c-Myc diluted in PBS. Incubate with continual inversion for 60 minutes.
3. Centrifuge for 1 minute at 12,000 × *g*. Then wash twice with 1 mL RIPA buffer at 4 °C.
4. Add 0.1-1.0 mL of cell extract containing the c-Myc-tagged protein to the beads (see **Note**). Incubate from 2 hours to overnight at 4 °C, with continual inversion.

   Note: The amount of cell extract required depends on the level of expression of the tagged protein and the specific application.
5. Separate the beads by centrifugation and remove the supernatant.
6. Wash the beads five times with 1 mL PBS each. Vortex (mix) the beads in the PBS. Then separate the beads by brief centrifugation.
7. Resuspend the pellet in 25 µL 2× SDS-PAGE sample buffer. Boil the sample for 5 minutes and centrifuge. The sample is ready to be loaded on a SDS-PAGE gel.

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