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ProductInformation

MONOCLONAL ANTI-c-MYC
Purified Mouse Immunoglobulin
Clone 9E10

Product Number **M 4439**

Product Description
Monoclonal Anti-c-Myc (mouse IgG1 isotype) is derived from the 9E10 hybridoma, produced by fusion of mouse myeloma cells and splenocytes from BALB/c mice. A synthetic peptide corresponding to residues 408-439 of the human $p62^{c\text{-}myc}$ protein conjugated to KLH was used as immunogen.[1] The isotype was determined using the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-c-Myc recognizes an epitope located within the sequence EQKLISEEDL (residues 410-419) of the product of the human oncogene *c-myc,* known as the c-Myc tag.[2] 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 (Product No. M 2435).[3]

Since the antibody reacts with both components of the $p62^{c\text{-}myc}$-$p64^{c\text{-}myc}$ doublet, by immunoblotting,[1,2] the antibody is also useful in immunohistochemical labeling of the c-Myc oncoprotein in formalin-fixed paraffin-embedded tissue sections, applying light,[5] and electron microscopy.[5,6] 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.

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. In addition, it allows characterization of newly identified, low abundance or poorly immunogenic proteins when protein specific antibodies are not available.[3,4]

The human *c-myc* proto-oncogene is the human cellular homologue of the avian *v-myc* gene found in several leukemogenic retroviruses.[1,7] Increased expression of the cellular oncogene *c-myc* has been described in a variety of human tumors, occurring by several mechanisms, including gene amplification and chromosomal translocation.[8] The gene encodes a polypeptide with predicted molecular weight of 49 kDa but showing aberrant electrophoretic mobility on polyacrylamide gel electrophoresis to give an apparent molecular weight of around 62 kDa ($p62^{c\text{-}myc}$).[6] $p62^{c\text{-}myc}$ is associated mainly with the cell nucleus, where it exerts its normal and oncogenic functions.[1,8]

Reagents
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: Approx. 2 mg/ml.

Precautions and Disclaimer
Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

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.

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: In order to obtain the best results using different techniques and preparations we recommend determining the optimal working dilution by titration.

Procedure

Procedure for Immunoblotting

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, Product No. D 8537) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20 (Product No. P 3563).
5. Incubate the membrane with Monoclonal Anti-c-Myc as the primary antibody for 60-120 min using an optimized concentration in PBS containing 0.05% Tween 20.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20.
7. Incubate the membrane with anti-mouse IgG Peroxidase conjugate (Product No. A 9917, A 3682, or A 2304) or with anti mouse Fab Alkaline Phosphatase conjugate (Product No. A 1293, A 2179 or A 1682) as the secondary antibody, at the recommended concentration in PBS containing 0.05% Tween 20. Incubate 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 containing 0.05% Tween 20.
9. Treat the membrane with a peroxidase or an alkaline-phosphatase substrate.

Procedure for Indirect Immunofluorescent Staining of Cultured Cells

All incubation steps should be performed at room temperature (except steps 1 and 3).

1. Grow transfected cultured cells expressing a c-Myc-tagged protein on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS.
3. Fix the cells with –20 °C methanol (10 minutes) and then with –20 °C acetone (1 minute).
4. Wash coverslips twice in PBS (5 minutes each wash).
5. Incubate coverslips cell-side-up with Monoclonal Anti-c-Myc in PBS containing 1% BSA. Incubate 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 (Product No. F 4018 or F 8771) 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 (Product No. P 3296) for 1 min at 12,000 × *g*, and 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 1mM EDTA) at 4 °C.
2. Add Monoclonal Anti c-Myc diluted in PBS, and incubate with continual inversion for 60 min.
3. Centrifuge for 1 min 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), and 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.

References

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