3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI- c-MYC
ALKALINE PHOSPHATASE CONJUGATE
CLONE 9E10

Product Number **A 5963**

Product Description

Monoclonal Anti-*c-myc* (mouse IgG1 isotype) is derived from the 9E10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized BALB/c mouse. 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 immunoglobulin fraction of antibody to *c-myc* is purified from ascites fluid and then conjugated to calf intestinal alkaline phosphatase using glutaraldehyde.

Monoclonal Anti-c-Myc, Alkaline Phosphatase conjugate recognizes an epitope located within the sequence EQKLISEEDL (residues 410-419) of the human oncogene product *c-myc.*[2] The antibody reacts with both components of the $p62^{c\text{-}myc}$-$p64^{c\text{-}myc}$ doublet, applying immunoblotting.[1,2] It is useful in ELISA[1] and in immunohistochemical labeling of *c-myc* oncoprotein in formalin-fixed paraffin-embedded tissue sections. Significant improvement has been reported in the quality and localization of staining of the antibody in tissues that have been treated by a rapid fixation, compared with routinely handled specimens.[3] Frozen sections post-fixed in acetone also retain some immunoreactivity with the antibody.[3] The antibody cross-reacts with human[1-11] $p62/64^{c\text{-}myc}$, 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.

Carcinogenesis is known to involve aberrant expression of genes involved in cell proliferation and differentiation. The *c-myc* gene has been implicated in the development of a number of neoplasms in a variety of avian and mammalian species.[1,11] The human *c-myc* protooncogene is the cellular homolog of the avian *v-myc* gene found in several leukemogenic retroviruses. Increased expression of the cellular oncogene *c-myc* has been described in a variety of human tumors, occurring by several different mechanisms, including gene amplification and chromosomal translocation.[3] 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 ($p62^{c\text{-}myc}$).[12] $p62^{c\text{-}myc}$ is associated mainly with cell nuclei, where it exerts its normal and oncogenic functions.[1] Immunohistochemical studies have shown an elevated level of c-myc protein in malignant tissues when compared with normal tissue, but with the unexpected finding of a cytoplasmic accumulation of the protein in these tumors.[3,11]

The sequence of the human *c-myc* gene (EQKLISEEDL) recognized by the 9E10 monoclonal antibody has become an important research tool in molecular biology. Recombinant DNA technology enables the insertion of genes of interest to specific sequences that can provide 'affinity handles' and thus enable the selective identification and purification of the protein of interest. It has been reported that the addition of the EQKLISEEDL sequence as a tag creates a stable fusion product that does not appear to interfere with the bioactivity of the protein or its biodistribution. The expression of polypeptides in-frame with the c-myc sequence allows for their detection, isolation and affinity purification.[2,5,6] Monoclonal antibody reacting specifically with c-myc may be useful in various immunotechniques, to study both endogenous c-myc or recombinant c-myc-tagged proteins.

Reagent

Monoclonal Anti-c-Myc, Alkaline Phosphatase conjugate is provided as a solution in 0.05 M Tris buffer pH 8.0, containing 1 % BSA, 1 mM $MgCl_2$, 50 % glycerol and 15 mM sodium azide.

Specific Antibody Concentration: At least 1 mg/ml

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

Storage/Stability
For continuous use and extended storage, store at 2 °C to 8 °C. **Do not freeze**. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
A minimum working dilution of 1:100 is determined by immunoblotting, using a recombinant c-Myc tagged fusion protein and BCIP/NBT substrate.

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

Procedure
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 to 20 µg of total lysate protein per lane. The amount of lysate to be loaded per lane depends on the level of protein expression and may vary between experiments.
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 Anti-c-Myc Alkaline Phosphatase conjugate using an optimized concentration in PBS containing 1 % bovine serum albumin (BSA, Product No. A 9647) for two hours.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20.
7. Treat the membrane with an alkaline phosphatase substrate (e.g. BCIP/NBT, Product No. B 1911).

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