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Product Information

Monoclonal Anti-c-Myc–Cy3™
Clone 9E10

Catalog Number **C6594**

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 using Protein A and then conjugated to Cy3. The conjugate is purified by gel filtration and contains no detectable free Cy3.

Monoclonal Anti-c-Myc–Cy3 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] Immuno-histochemical 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 attachment 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 poly-peptides 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

Supplied as a solution in 0.01 M PBS, pH 7.4, containing 1 % BSA and 15 mM sodium azide.

Specific Antibody Concentration:	0.5 to 2 mg/ml
Molar ratio F/P of the product:	3.0 to 9.0

Spectral Characteristics of Cy3:[13]
Absorbance Max = 552 nm
Emission Max = 570 nm

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use and extended storage, store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Solutions at working dilution should be discarded if not used within 12 hours.

Product Profile
A minimum working dilution of 1:50 is determined by direct immunofluorescent labeling of formalin-fixed paraffin-embedded human colon carcinoma tissue.

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

Procedure
Direct Immunofluorescent Staining of Cultured Cells
All incubation steps should be performed at room temperature (except step 3).
1. Grow transfected cultured cells expressing c-Myc-fusion protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS, Catalog No. D8537.
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). Note: Blocking with PBS containing 1% BSA, Catalog No. A9647, for 10 minutes at room temperature followed by draining prior to step 5 may minimize non-specific adsorption of the antibody.
5. Incubate coverslips cell-side-up with Monoclonal Anti-c-Myc-Cy3 in PBS for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
8. Examine using a fluorescence microscope with appropriate filters.

Note: It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary antibody. The ideal negative control reagent is a Cy3 conjugated mouse monoclonal antibody or myeloma protein. It should be isotype-matched, F/P molar ratio-matched, not specific for the tested preparation, and of the same concentration as the tested antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of preparation under study and the sensitivity of the instrument used. For fluorescent analysis of preparation expressing Fc receptors, the use of isotype-matched negative contols is mandatory.

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