

Product Information

MONOCLONAL ANTI-*c-myc*

Clone 9E10

Mouse Ascites Fluid

Product Number **M 5546**

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 immunized BALB/c mice. A synthetic peptide corresponding to residues 408-439 of the human p62^{c-myc} protein, conjugated to KLH, was used as immunogen.¹ The isotype is determined using 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 in the amino acid sequence EQKLISEEDL (residues 410-419) of the human oncogene product *c-myc*.² The antibody reacts with both components of the p62^{c-myc}-p64^{c-myc} doublet in immunoblotting.^{1,2} It may be used in the immunoprecipitation of *c-myc*-tagged fusion proteins.^{3,4} However, it does not immunoprecipitate native or denatured *c-myc* protein from cell lysate.¹ The antibody may also be used in immunohistochemical labeling of *c-myc* oncoprotein in formalin-fixed, paraffin-embedded tissue sections, and in light⁵⁻¹⁰ and electron^{10,11} microscopy. Significant improvement has been reported in the quality and localization of staining in tissues that have been treated by a rapid fixation, compared with routinely handled specimens.⁹ Frozen sections post-fixed in acetone also retain some immunoreactivity with the antibody.⁹ Additional applications of the product include ELISA,¹ immunocytochemistry of cells that have been transfected² or microinjected¹¹ with *c-myc*-tagged protein encoding vectors, as well as the detection of *c-myc*-tagged antibodies by electron microscopy.¹² The antibody cross-reacts with human¹⁻¹¹ p62/64^{c-myc}, but does not recognize the chicken p110^{gag-myc} protein present in MC29 virus-transfected quail fibroblasts, nor does it react with the mouse p64/66^{c-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. One gene implicated in the development of a number of neoplasms in a variety of avian and mammalian species¹ is the oncogene *c-myc*.¹⁰ The human *c-myc*

proto-oncogene is the human 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.⁹ 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-myc}).¹³ p62^{c-myc} is associated mainly with the nuclei of cells, where it exerts its normal and oncogenic functions.¹ Indeed, 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.^{9,10} The human *c-myc* gene has provided a research tool in other fields. Thus for instance, recombinant DNA technology enables the insertion of genes of interest to specific sequences of genes, which can provide 'affinity handles' designed to bind specific matrices. It has been reported that the addition of *c-myc* sequences as a tag, creates a stable fusion product that does not appear to interfere with the bioactivity of the protein or with the biodistribution of the *c-myc* tagged product. The expression of polypeptides in-frame with *c-myc* sequences allows for their detection, isolation and affinity purification.²⁻⁴ Monoclonal antibody reacting specifically with *c-myc* may be used in various immunotechniques, to study the prevalence of the protein product of the *c-myc* oncogene in cells and tissues at the light microscope and ultrastructural levels.⁵⁻¹² It may also be used to identify the expression of a *c-myc* fusion protein in bacterial lysates or in cells and tissues transfected or microinjected with a *c-myc* fusion protein encoding vectors.

Reagents

The product is provided as ascites fluid with 15 mM sodium azide as a preservative.

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, store at 2-8 °C for a maximum of one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

1. A minimum working dilution of 1:100 is determined by immunoblotting of *c-myc*-tagged fusion protein.
2. A minimum working dilution of 1:500 is determined by indirect immunoperoxidase labeling of formalin-fixed, paraffin-embedded human colon carcinoma tissue.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

References

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