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ProductInformation

Monoclonal Anti-c-Myc FITC conjugate

Clone 9E10 Purified Mouse Immunoglobulin

Product Number F 2047

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-myc} protein conjugated to KLH was used as the immunogen. The antibody is isolated from ascites fluid and conjugated to fluorescein isothiocyanate isomer I (FITC).

Monoclonal Anti-c-Myc, FITC conjugate recognizes the c-Myc tag sequence on c-Myc tagged fusion proteins when expressed N- or C-terminal to the fusion protein. The antibody reacts specifically with c-Myc tagged fusion proteins by immunocytochemistry.

An epitope located within amino acids 410-419, containing the sequence EQKLISEEDL of human c-Myc has been widely used as a tag in many expression vectors, enabling the expression of proteins as c-Myc tag fusion proteins.² 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.²⁻⁵ Anti-c-Myc, FITC conjugate, is useful for detection and localization of c-Myc fusion proteins by immunofluorescence.

Reagent

The product is provided as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Specific Antibody concentration: minimum 0.8 mg/ml. F/P Molar Ratio: 6-8.

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 hazardous 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. 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. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A concentration of 5.0 $\mu g/ml$ of the antibody detects c-Myc tagged fusion proteins in transfected mammalian cells by immunofluorescence.

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

Procedure for Direct Immunofluorescent Staining of Cultured Cells

- Grow transfected cultured cells expressing c-Myc fusion protein of choice on sterile coverslips at 37 °C.
- 2. Wash the cells briefly in PBS (PBS, Product No. P 3813).
- 3. Fix the cells for 10 minutes with 3% paraformaldehyde, rinse briefly, and immediately permeabilize the cells by incubating 2 to 10 minutes with 0.5% Triton® X-100 in PBS.
- 4. Wash the coverslips twice in PBS (5 minutes each wash).
- Incubate the coverslips cell-side-up with Antic-Myc, FITC conjugate in PBS containing 1% BSA (BSA, Product No. A 7906) for 1 hour. Note: The addition of BSA helps reduce nonspecific staining.
- 6. Wash the slides three times in PBS (5 minutes each wash).
- 7. Place one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles. Examine using a fluorescence microscope with appropriate filters.

References

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- Olins, P.O., and Lee, S.C., Curr. Opin. Biotechnol., 4, 520-525 (1993).
- 4. Uhlen, M., and Moks, T., Methods Enzymol., **185**, 129-143 (1990).
- 5. Kolodziej, P.A., and Young, R.A., Methods Enzymol., **194**, 508-519 (1991).

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