

Product Information

Monoclonal Anti-c-Myc-Biotin, clone 9E10

produced in mouse, purified immunoglobulin

Catalog Number **B7554**

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 immunized with a synthetic peptide corresponding to residues 408-439 of the human p62^{c-myc} protein conjugated to KLH.¹ The antibody is isolated from ascites fluid and conjugated to biotin.

Anti-c-Myc recognizes the c-Myc tag sequence on c-Myc tagged fusion proteins when expressed N- or C-terminal to the fusion protein. Anti-c-Myc- Biotin reacts specifically with c-Myc tagged fusion proteins by immunoblotting, and the reaction is inhibited by the c-Myc peptide, Catalog Number M2435. Under certain experimental conditions, endogenous biotin-containing proteins may be detected at about 72, 78 and 130 kDa.^{2,3}

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.⁴ 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.⁴⁻⁶

Reagent

Supplied in 0.01 M phosphate buffered saline pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Antibody concentration: Minimum 0.8 mg/ml.

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, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or 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

Immunoblotting: a working antibody concentration of 0.05 to 0.1 µg/ml detects c-Myc tagged fusion proteins in extracts of transfected 293T cells or transfected *E.coli* cells.

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

Procedure

Procedure for Immunoblotting

All incubation steps should be performed at room temperature.

Note: The avidin-biotin based system is extremely sensitive. As such, non-specific background (i.e. endogenous biotin) may appear. We recommend the following protocol for preventing detection of non-specific bands.

1. Separate c-Myc tagged proteins from sample lysates using a standard 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 and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of PBS containing 10% BSA and 0.1% TWEEN® 20. Incubate from 1 hour at room temperature to overnight at 4 °C (PBS, Catalog No. P3813; BSA, Catalog No. A7906; TWEEN 20, Catalog No. P1379).

4. Wash the membrane three times for 10 minutes each in PBS containing 0.1% TWEEN 20.
5. Incubate the membrane with an optimized concentration of Anti-c-Myc, biotin conjugate as the primary antibody diluted in PBS containing 0.1% TWEEN 20.
6. Wash the membrane three times for 10 minutes each in PBS containing 0.1% TWEEN 20.
7. Incubate the membrane for 30 minutes with ExtrAvidin[®]-Peroxidase, Catalog No. E2886, as the secondary reagent in PBS containing 0.1% TWEEN 20 and 1% BSA. Note: The recommended starting dilution for E2886 is 1:15,000; adjust the ExtrAvidin-Peroxidase concentration as necessary to maximize detection sensitivity and minimize background.
8. Wash the membrane three times for 10 minutes each in PBS containing 0.1% TWEEN 20.
9. Treat the membrane with a peroxidase substrate.

References

1. Evan, G. I., et al., *Mol. Cell Biol.*, **5**, 3610-3616 (1985).
2. Chandler, C. S., and Bailard, F. J., *Biochem. J.*, **237**, 123-130 (1996).
3. Vaitaitis, G. M., et al., *Biotechniques*, **26**, 854-858 (1999).
4. Pelengaris, S., et al., *Curr. Opin. Genet. Dev.*, **10**, 100-105 (2000).
5. Jarvik, W., and Telmer, C. A., *Annu. Rev Genet.*, **32**, 601-618 (1998).
6. Olins, P. O., and Lee, S. C., *Curr. Opin. Biotechnol.*, **4**, 520-525 (1993).

TWEEN is a registered trademark of Croda International PLC

DS,KAA,PHC 12/12-1