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Product Information

Anti-c-Myc
produced in rabbit, affinity isolated antibody

Catalog Number **C3956**

Product Description

Anti-c-Myc is produced in rabbit using as immunogen a peptide corresponding to amino acids 408-425 of the human c-*m*yc proto-oncogene, conjugated to maleimide-activated KLH through a C-terminal added cysteine residue. The antibody is affinity-purified on the immobilized immunizing peptide.

Anti-c-Myc recognizes the c-Myc tag sequence (EQKLISEEDL) 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 immunoblotting. Reaction of the antibody in immunoblotting is inhibited by the c-Myc Peptide, Catalog Number M2435. Anti-c-Myc immunoprecipitates c-Myc-tagged fusion proteins from cell lysates, and by indirect immunofluorescence stains transiently transfected cells expressing c-Myc tagged proteins. The antibody has not been tested to determine if it recognizes endogenous c-Myc.

The human *c-myc* proto-oncogene is the human cellular homologue of the avian *v-myc* gene found in several leukemogenic retroviruses.[1-3] Increased expression of the cellular oncogene c-*myc* has been described in a variety of human tumors, occurring by several mechanisms, including gene amplification and chromosomal translocation.[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, they allow 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% bovine serum albumin and 15 mM sodium azide.

Antibody concentration: 0.5-0.8 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: 0.5-1 µg/ml of the antibody can detect c-Myc fusion proteins in cell extracts from transfected cultures as well as bacterial lysates.

Immunocytochemistry: 5-10 µg/ml is the recommended antibody concentration

Indirect imunofluorescence: 5-10 µg/ml of the antibody detects c-Myc fusion proteins in methanol-acetone fixed transiently transfected cells.

Immunoprecitation, 1-2 µg of the antibody can immunoprecipitate a c-Myc fusion protein from transfected mammalian cell lysates or bacterial extracts.

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

Procedures

A. Procedure for Immunoblotting

Note: Perform the entire procedure 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 2.5-20 μg total lysate protein per lane.
   Note: The amount of lysate to be loaded depends on the level of protein expression and may vary between experiments.
   Transfer proteins from the gel to a nitrocellulose membrane.
2. Block the membrane using a solution of 5 % non-fat dry milk in PBS at room temperature for 1 hour.
3. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % TWEEN® 20.
4. Incubate the membrane with Anti-c-Myc antibody as the primary antibody diluted to ~1.0 μg/ml in PBS containing 0.05 % TWEEN with agitation for 120 minutes.
5. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % TWEEN 20.
6. Incubate the membrane with Anti-Rabbit IgG Peroxidase, Catalog Number A0545, as the secondary antibody at the recommended concentration in PBS containing 0.05 % TWEEN 20. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
7. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN 20.
8. Incubate the membrane with a peroxidase substrate.

B. Procedure for Indirect Immunofluorescent Staining of Cultured Cells

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.
3. Fix the cells with –20 °C methanol (10 minutes) and then with –20 °C acetone (1 minute).
4. Wash specimens twice in PBS (5 minutes each wash).
5. Incubate specimens cell-side-up with Anti-Myc antibody as primary antibody diluted to 5 μg/ml to 10 μg/ml in PBS containing 1% BSA. Incubate at room temperature for 1 hour.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate specimens cell-side-up with Anti-Rabbit IgG-FITC, Catalog Number F9887, as the secondary antibody at the recommended dilution in PBS containing 1% BSA. Incubate at room temperature for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Coverslip with aqueous mounting medium and examine using a fluorescence microscope with appropriate filters.
   Note: Blocking with PBS containing 1 % BSA for 10 minutes at room temperature prior to step 5 may minimize non-specific adsorption of the antibodies.

C. Procedure for Immunoprecipitation

1. Centrifuge 40 µL of a 1:1 suspension of Protein A - Agarose Fast Flow, Catalog Number P3476, for 1 min. 12,000 x *g*, and then wash twice with 1 ml RIPA buffer (50 mM Tris base, 0.25% w/v deoxycholate, 1% IGEPAL® CA-630, 150 mM NaCl, 1mM EDTA, pH 7.4) at 4 ºC.
2. Add Anti-c-Myc antibody diluted to 0.5 μg/ml to 1.0 μg/ml in PBS, and incubate by swinging head-over-tail for 1 hour at room temperature.
3. Centrifuge 1 min 12,000 X *g*, wash twice with 1 ml RIPA at 4 ºC.
4. Add 0.1-1.0 ml of cell extract containing c-Myc tagged protein to the beads (see note), and incubate from 2 hours to overnight at 4 ºC, while swinging head-over tail.
   Note: The amount of cell extract depends on the level of expression of the tagged protein and the specific application.
5. Spin down beads; remove supernatant.
6. Wash beads four times with 1ml RIPA buffer and once with PBS by vortex and short spin.
7. Resuspend pellet in 25 µL 2X SDS-PAGE sample buffer. Boil sample for 5 min and spin down. The sample is ready to be loaded on an SDS-PAGE gel.

References

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