Product Information

Anti-c-Myc

Produced in rabbit, affinity isolated antibody, buffered aqueous solution

C3956

Product Description

The human c-*myc* proto-oncogene is the human cellular homologue of the avian v-*myc* gene that is found in several leukemogenic retroviruses.[1-3] Increased expression of the cellular oncogene c-*myc* has been described in various human tumors, occurring by several mechanisms, which include 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-tagged fusion proteins.[4] Epitope tags provide a method to localize gene products in various cell types. Such tags allow characterization of newly identified, low abundance or poorly immunogenic proteins when protein specific antibodies are not available.[4-6]

Anti-c-Myc is produced in rabbit, using as the immunogen a peptide that corresponds to amino acids 408-425 of the human c-*myc* 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 (Cat. No. M2435). Anti-c-Myc immunoprecipitates c-Myc-tagged fusion proteins from cell lysates. By indirect immunofluorescence, it stains transiently transfected cells that express c-Myc tagged proteins. The antibody has not been tested to determine if it recognizes endogenous c-Myc.

Several theses[7-8] and dissertations[9-18] cite use of this C3956 product in their protocols.

Reagent

This product is 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

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.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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

Recommended antibody concentration: 5-10 µg/mL

Indirect immunofluorescence

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: To obtain best results and assay sensitivity in different techniques and preparations, we recommend determining optimal working dilutions by titration.

Procedure

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. 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® 20, 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 (Cat. No. A0545) as the secondary antibody, at the recommended concentration in PBS containing 0.05% TWEEN® 20, 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.

Procedure for Indirect Immunofluorescent Staining of Cultured Cells

1. Grow transfected cultured cells that express the 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 the specimens twice in PBS (5 minutes each wash).
5. Incubate the specimens cell-side-up with Anti-Myc antibody as the primary antibody diluted to 5 µg/mL to 10 µg/mL in PBS containing 1% BSA, at room temperature for 1 hour.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate the specimens cell-side-up with Anti-Rabbit IgG-FITC (Cat. No. F9887) as the secondary antibody at the recommended dilution in PBS containing 1% BSA, at room temperature for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Coverslip the specimens with aqueous mounting medium. 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.

Procedure for Immunoprecipitation

1. Centrifuge 40 µL of a 1:1 suspension of Protein A-Agarose for 1 minute at 12,000 x *g*. Then wash twice with 1 mL RIPA buffer (50 mM Tris base, 0.25% w/v deoxycholate, 1% IGEPAL® CA-630, 150 mM NaCl, 1 mM EDTA, pH 7.4) at 4 °C.
2. Add Anti-c-Myc antibody, diluted to a range of 0.5 µg/mL to 1.0 µg/mL in PBS, to the Protein A-Agarose suspension. Incubate by swinging head-over-tail for 1 hour at room temperature.
3. Centrifuge 1 minute at 12,000 × *g*. Wash twice with 1 mL RIPA at 4 °C.
4. Add 0.1-1.0 mL of cell extract containing c-Myc-tagged protein to the beads (see **Note**). Incubate from 2 hours to overnight at 4 °C, 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 the beads. Remove supernatant.
6. Wash beads four times with 1 mL 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 minutes and spin down. The sample is ready to be loaded on an SDS-PAGE gel.

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