

**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. <sup>1-3</sup> 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. <sup>3</sup>

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.<sup>4</sup> 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.<sup>4-6</sup>

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<sup>7-8</sup> and dissertations<sup>9-18</sup> 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**

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 $0.5\text{--}1~\mu\text{g/mL}$  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

 $1\mbox{-}2~\mu g$  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.

- 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.
- 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  $\sim 1.0~\mu g/mL$  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.
- Incubate the membrane with a peroxidase substrate.

# Procedure for Indirect Immunofluorescent Staining of Cultured Cells

- 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).
- 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

- 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  $0.5~\mu g/mL$  to  $1.0~\mu g/mL$  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.
- 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  $\mu$ L 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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