

Product Information

Anti-c-Myc-Peroxidase antibody produced in rabbit

Affinity isolated antibody, buffered aqueous solution

A5598

Product Description

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

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 An epitope located within amino acids 410-419 of human c-Myc, containing the sequence EQKLISEEDL, has been widely used as a tag in many expression vectors, to enable the expression of recombinant proteins as c-Myc-tagged fusion proteins.⁴

Anti-c-Myc, peroxidase conjugate, is a buffered aqueous solution containing affinity-purified anti-c-Myc antibody conjugated to horseradish peroxidase (HRP). The anti-c-Myc antibody is developed in rabbit using a peptide corresponding to amino acids 408-425 of the human *c-myc* proto-oncogene.

Anti-c-Myc, peroxidase conjugate, recognizes the c-Myc tag sequence, EQKLISEEDL, that has been expressed at either the amino- or the carboxyl-terminus of a c-Myc tagged fusion protein.¹ The antibody reacts specifically with c-Myc tagged fusion proteins by immunoblotting. Staining of the antibody in immunoblotting is specifically inhibited by the c-Myc peptide (Cat. No. M 2435).

Reagent

Anti-c-Myc, Peroxidase conjugate, is supplied as a solution in 0.01 M sodium phosphate buffered saline (PBS), pH 7.4, containing 0.05% MIT.

Antibody concentration: 1.8-4.0 mg/mL

Molar ratio of antibody/enzyme: 0.8-1.5

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 a frost-free freezer is **not** recommended.
- If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.
- Working dilutions should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:5000 is determined by immunoblotting of an *E. coli* extract which expresses a recombinant c-Myc tagged fusion protein, and by subsequently using a chemiluminescent substrate.

Note: To obtain the best results using different techniques and preparations, we recommend determining the optimal working dilution by titration.

Procedure

Procedure for Immunoblotting

Note: All incubation steps should be performed at room temperature.

1. Separate c-Myc-tagged proteins from the sample extract using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load adequate bacterial or mammalian lysate expressing the c-Myc-tag fusion protein (2-20 µg).
 - The amount of extract to be loaded per lane depends on the level of protein expression.
 - Thus the optimum loading may vary between preparations.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk in phosphate buffered saline (PBS, such as Cat. No. D8537) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween® 20 (such as Cat. No. P3563).
5. Incubate the membrane with an optimized concentration of Anti-c-Myc, Peroxidase conjugate, for 60-120 minutes in PBS containing 0.05% Tween® 20.
6. Wash the membrane three times for 15 minutes each in PBS containing 0.05% Tween® 20.
7. Treat the membrane with a peroxidase substrate.

References

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A5598dat Rev 08/21 NV,RC,GCY

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