

Anti-c-myc

Mouse monoclonal antibody (clone 9E10), IgG₁, κ

Cat. No. 11 667 149 001
Cat. No. 11 667 203 001

200 μg (lyophilized)
5 mg (1 ml solution)

 **Version 06**
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Store at –15 to –25°C

1. What this Product Does

Contents

The antibody is supplied as either a white lyophilizate containing 200 μg anti-c-myc monoclonal antibody in phosphate-buffered saline containing 0.2% gelatin for stability, or as a liquid consisting of 5 mg anti-c-myc monoclonal antibody in phosphate-buffered saline (PBS), pH 7.4.

Storage and Stability

Lyophilized and liquid anti-c-myc antibody preparations are stable until the control date printed on the vial when stored at –15 to –25°C. For liquid and reconstituted samples, avoid repeated freezing and thawing. For storage, prepare convenient aliquots, and freeze them at –15 to –25°C.

- 🕒 Lyophilized Anti-c-myc is shipped at ambient temperature.
- 🕒 Liquid Anti-c-myc antibody is shipped on dry ice.

Application

Anti-c-myc (clone 9E10) is used for the immunochemical detection of native human c-myc protein and recombinant “epitope tagged” proteins that contain the c-myc epitope in western and dot blots, immunocytochemistry, and immunoprecipitation.

Product Characteristics

Specificity	Anti-c-myc recognizes the 9E10 epitope (sequence EQKLISEEDL), which was derived from the human c-myc protein (1). The monoclonal antibody against the c-myc epitope is well characterized (1) and does not cross-react with other cellular proteins. The antibody recognizes its antigenic determinant even when the c-myc peptide epitope is introduced into unrelated recombinant proteins by a technique known as “epitope tagging” (6).
Clone	9E10 (1)
Subtype	Mouse IgG ₁ κ
Purity	Anti-c-myc monoclonal antibody is ≥90% pure as determined by HPLC.

2. How to Use this Product

2.1 Before You Begin

Working Concentration

For the lyophilized anti-c-myc preparation, prepare a concentrated stock solution (0.4 mg/ml) by dissolving the entire lyophilized anti-c-myc preparation (200 μg) in 500 μl phosphate-buffered saline (PBS).

For most applications, dilute the anti-c-myc solution (either 5 mg/ml or 0.4 mg/ml) in an appropriate buffer (*e.g.*, Tris-buffered saline containing 0.1% Tween 20) to a concentration range of 1–10 μg/ml. Determine optimal dilution buffer and dilution conditions for each specific application and method.

Molecular Biology

Before using anti-c-myc to analyze the product of your target gene, incorporate the 30-base DNA sequence that encodes the c-myc epitope into the target gene sequence by one of the following methods:

- Prepare oligonucleotide linkers that can encode the c-myc epitope, and clone the linkers into the target gene at the desired N-terminal, C-terminal, or internal site (2).
- Insert the c-myc peptide coding sequence into the target gene by oligonucleotide-mediated site-directed mutagenesis (3).

2.2 Procedure

Western Blot

The following method has been developed for the anti-c-myc antibody, but may have to be optimized for specific experimental systems:

- 1 Lyse cells that contain the c-myc epitope, and prepare the lysates for SDS gel electrophoresis.
- 2 Perform SDS gel electrophoresis on cell lysates.
- 3 Perform western blot transfer to a PVDF membrane in transfer buffer containing 20% methanol, 24 mM Tris base, 194 mM glycine.
- 4 Transfer the membrane to a tray, and incubate the membrane for 1 hour at +15 to +25°C (or O/N at +2 to +8°C) with a 1:10 dilution of Western Blocking Reagent* in phosphate buffered saline with 0.1% Tween 20, pH 7.5 (PBST).
- 5 Wash the membrane three times with PBST.
- 6 Dilute anti-c-myc antibody concentrate to 1 μg/ml in a 1:20 dilution of Western blocking reagent. Incubate the membrane with this diluted anti-c-myc antibody for 1–2 hours at +15 to +25°C with gentle rotation.
- 7 Wash the membrane three times with 1× Wash Buffer (as in Step 5).
- 8 Dilute goat anti-mouse IgG (H+L) HRP conjugate 1:4000 into a 1:20 dilution of Western blocking reagent. Incubate the membrane with this diluted antibody solution for 1 hour at RT with gentle rotation.
- 9 Wash the membrane three times with 13 Wash Buffer (Step 5).
- 10 Prepare Lumi-Light Western Blotting Substrate according to pack insert instructions. Apply Lumi-Light Reagent to the membrane.
- 11 Expose the membrane to X-ray film. For a 1 minute substrate development, initially perform a 1–5 minute film exposure. The conditions for development and exposure may vary.

3. Additional Information on this Product

Background Information

Anti-c-myc was originally developed to study c-myc, one of a family of nuclear proteins that has been found in several types of human tumors (2,4). However, subsequent studies (5) have used anti-c-myc to detect and purify proteins whose DNA coding sequences have been fused to the coding sequence of the c-myc epitope by recombinant DNA techniques. Such epitope tagging studies are useful for:

- determining size, intracellular localization, and abundance of proteins produced by newly discovered genes
- tracking intra compartmental sorting of a family of proteins
- analyzing the function of individual protein domains
- confirming post-translational modification of proteins
- following the fate of transfected proteins
- monitoring receptor binding and internalization of exogenous proteins
- discovering the function of proteins that are difficult to purify or share epitopes with a number of other proteins
- studying the effects of over-expressed proteins on cellular processes.

Preparation

Clone 9E10 was obtained by immunizing BALB/c mice with the peptide AEEQKLISEEDLLRKRREQLKHKLEQLRNSCA, which corresponds to amino acid residues 408-439 in the human c-myc protein (1). Spleen cells were then fused with SP2/0 myeloma cells to produce the 9E10 hybridoma clone. Antibody was produced by cells cultured in fetal calf serum-supplemented culture medium. After purification by Protein G, the antibody is either lyophilized in the 200- μ g pack size or stored as a 5-mg liquid pack size.

Quality

In the procedure described above, the anti-c-myc antibody is tested for functionality and purity relative to a reference standard to confirm the quality of each new reagent lot.

A cell lysate containing the c-myc tagged protein is resolved by SDS-PAGE and transferred to a PVDF membrane. When incubated with the blot membrane at a concentration of 1.0 μ g antibody/ml, the anti-c-myc antibody binds specifically to the c-myc tagged protein. The antigen/antibody complex on the membrane is visualized with an anti-[mouse IgG]-horseradish peroxidase (HRP) conjugate and a chemiluminescent HRP substrate.

References

- 1 Evan, G.I., Lewis, G.K., Ramsay, G. and Bishop, J.M. (1985) *Mol. Cell. Biol.* **5**: 3610-3616.
- 2 Nisen, P.D., Zimmerman K.A., Cotter, S.V., Gilbert, F. and Alt, F.W. (1986) *Cancer Res.* **46**: 6217-6222.
- 3 Harlow, E. and Lane, D., *Antibodies -A Laboratory Manual*, 1988, p. 447.
- 4 Alitalo, K., Schwab, M., Lin, C.C., Varmus, H.E., and Bishop, M. (1983) *Natl. Acad. Sci. USA* **80**: 1707-1711.
- 5 Cravchik, A. and Matus, A. (1993) *Gene* **137**: 139-143.
- 6 Kolodziej, P.A. and Young, R.A. (1991) *Methods Enzymol.* **194**: 508-519.

4. Supplementary Information

4.1 Text Conventions

To make information consistent and easy-to-read, the following text conventions are used in this Instruction Manual:

Text Convention	Use
Numbered instructions labeled 1 , 2 , etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols

Symbols are used in this Instruction Manual to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

Abbreviations

D	Aspartic acid	K	Lysine
I	Isoleucine	Q	Glutamine
E	Glutamic acid	S	Serine
L	Leucine	A	Alanine
R	Arginine	N	Asparagine
H	Histidine	C	Cysteine

4.2 Changes to Previous Version

- Editorial changes

4.3 Ordering Information

Roche Diagnostics offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, <http://lifescience.roche.com>

Further epitope tagging related products can be found under <http://lifescience.roche.com/sis/proteomicscience/>

Product	Pack Size	Cat No.
Anti-HA (12CA5)	200 μ g	11 583 816 001
	5 mg (1ml)	11 666 606 001
Anti-HA-Biotin	100 μ g (500 μ l)	11 666 851 001
Anti-HA-Fluorescein	100 μ g (500 μ l)	11 666 878 001
Anti-HA High Affinity (3F10)	50 μ g	11 867 423 001
	500 μ g	11 867 431 001
Anti-HA-Biotin, High Affinity (3F10)	50 μ g	12 158 167 001
Anti-HA-Fluorescein, High Affinity (3F10)	25 μ g	11 988 506 001
Anti-HA-Peroxidase, High Affinity (3F10)	25 μ g	12 013 819 001
Anti-HA Affinity Matrix	1 ml	11 815 016 001
HA Peptide	5 mg	11 666 975 001
Protease/Phosphatase Inhibitor Tablets and Lysis Reagents		
cOplete	20 tablets in glass vials	11 697 498 001
	3 \times 20 tablets in glass vials	11 836 145 001
	20 tablets in <i>EASYpacks</i>	04 693 116 001
cOplete, Mini	25 tablets in a glass vial	11 836 153 001
	30 tablets in <i>EASYpacks</i>	04 693 124 001
cOplete, EDTA-free	20 tablets in a glass vial	11 873 580 001
	3 \times 20 tablets in glass vials	05 056 489 001
	20 tablets in <i>EASYpacks</i>	04 693 132 001
cOplete, Mini, EDTA-free	25 tablets in a glass vial	11 836 170 001
	30 tablets in <i>EASYpacks</i>	04 693 159 001
cOplete Lysis-B (2 \times) (for bacterial cell lysis)	1 kit (100 ml lysis reagent and 20 cOplete Protease Inhibitor Cocktail Tablets)	04 719 930 001
cOplete Lysis-B (2 \times), EDTA-free (for bacterial cell lysis)	1 kit (100 ml lysis reagent and 20 cOplete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 948 001

Product	Pack Size	Cat No.
cOmplete Lysis-M (for mammalian cell lysis) and 20 cOmplete Protease Inhibitor Cocktail Tablets)	1 kit (200 ml lysis reagent and 20 cOmplete Protease Inhibitor Cocktail Tablets)	04 719 956 001
cOmplete Lysis-M, EDTA-free (for mammalian cell lysis) and 20 cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets)	1 kit (200 ml lysis reagent and 20 cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 964 001
PhosSTOP	20 tablets in <i>EASYPacks</i>	04 906 837 001
	10 tablets in <i>EASYPacks</i>	04 906 845 001
Transfection Reagents		
X-tremeGENE HP DNA Transfection Reagent	1 trial pack 0.4 ml 1.0 ml 5 x 1 ml	06 365 752 001 06 366 244 001 06 366 236 001 06 366 546 001
Western Blotting Reagents		
Lumi-Light ^{PLUS} Western Blotting Kit (Mouse/Rabbit)	1 kit (1,000 cm ² membrane)	12 015 218 001
Lumi-Light Western Blotting Substrate	400 ml, (4000 cm ² membrane)	12 015 200 001
Lumi-Light ^{PLUS} Western Blotting Substrate	100 ml, (1,000 cm ² membrane)	12 015 196 001
Lumi-Film Chemiluminescent Detection Film	100 films (8 x 10 inches 20.3 x 25.4 cm)	11 666 657 001
PVDF Western Blotting Membranes	1 roll (30 cm x 3.00 m)	03 010 040 001
Western Blocking Reagent, Solution	100 ml (10 blots, 100 cm ²) 6 x 100 ml (60 blots, 100 cm ²)	11 921 673 001 11 921 681 001
Bovine Serum Albumin, Fraction V	50 g 100 g 500 g 1 kg	10 735 078 001 10 735 086 001 10 735 094 001 10 735 108 001
Detergents		
Triton X-100	5 x 10 ml	11 332 481 001
Tween 20	5 x 10 ml	11 332 465 001
Nonidet P40	5 x 10 ml	11 332 473 001
Buffers in a Box, Pre-mixed PBS Buffer, 10x	4 l	11 666 789 001
Immunoprecipitation Reagents		
Immunoprecipitation Kit (Protein G)	20 reactions	11 719 386 001
Protein G Agarose	2 ml 5 ml 15 ml	11 719 416 001 11 243 233 001 05 015 952 001

4.4 Trademarks

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