

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 05
Content version: July 2009

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.

* available from Roche Applied Science

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-µg 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 µg 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 Applied Science.

Symbols

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

Symbol	Description
③	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
- New layout

4.3 Ordering Information

Roche Applied Science 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, www.roche-applied-science.com.

Further epitope tagging related products can be found under http://www.roche-applied-science.com/sis/proteomicscience/prot_characterization/epitope_tagging.htm.

Product	Pack Size	Cat No.
Anti-HA (12CA5)	200 µg 5 mg (1ml)	11 583 816 001 11 666 606 001
Anti-HA-Biotin	100 µg (500 µl)	11 666 851 001
Anti-HA-Fluorescein	100 µg (500 µl)	11 666 878 001
Anti-HA-Rhodamine	100 µg (500 µl)	11 666 959 001
Anti-HA High Affinity (3F10)	50 µg 500 µg	11 867 423 001 11 867 431 001
Anti-HA-Biotin, High Affinity (3F10)	50 µg	12 158 167 001
Anti-HA-Fluorescein, High Affinity (3F10)	25 µg	11 988 506 001
Anti-HA-Peroxidase, High Affinity (3F10)	25 µg	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

cOmplete	20 tablets in glass vials 3 × 20 tablets in glass vials 20 tablets in EASYpacks	11 697 498 001 11 836 145 001 04 693 116 001
cOmplete, Mini	25 tablets in a glass vial 30 tablets in EASYpacks	11 836 153 001 04 693 124 001
cOmplete, EDTA-free	20 tablets in a glass vial 3 × 20 tablets in glass vials 20 tablets in EASYpacks	11 873 580 001 05 056 489 001 04 693 132 001
cOmplete, Mini, EDTA-free	25 tablets in a glass vial 30 tablets in EASYpacks	11 836 170 001 04 693 159 001
cOmplete Lysis-B (2×) (for bacterial cell lysis)	1 kit (100 ml lysis reagent and 20 cOmplete Protease Inhibitor Cocktail Tablets)	04 719 930 001

Product	Pack Size	Cat No.
cOmplete Lysis-B (2×), EDTA-free (for bacterial cell lysis)	1 kit (100 ml lysis reagent and 20 cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 948 001
cOmplete Lysis-M (for mammalian cell lysis)	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)	1 kit (200 ml lysis reagent and 20 cOmplete, EDTA-free Protease Inhibitor Cocktail Tablets)	04 719 964 001
PhosSTOP	20 tablets in EASYpacks	04 906 837 001
	10 tablets in EASYpacks	04 906 845 001
Transfection Reagents		
FuGENE® 6 Transfection Reagent	0.4 ml (120 transfections)	11 815 091 001
	1 ml (300 transfections)	11 814 443 001
	Multi-pack 5 × 1 ml (1,500 transfections) ¹⁾	11 815 075 001
	Mega-pack 5 × 1 ml (1,500 transfections)	11 988 387 001
	10 ml (3,000 transfections)	05 061 377 001
FuGENE® HD Transfection Reagent	0.4 ml (120 transfections)	04 709 691001
	1 ml (300 transfections)	04 709 705 001
	Trial pack	04 883 560 001
	Mega-pack 5 × 1 ml (1,500 transfections) ¹⁾	04 709 713 001
	10 ml (3,000 transfections)	05 061 369 001
¹⁾ The five vials are packaged together in one box with one pack insert.		
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 × 10 inches 20.3 × 25.4 cm)	11 666 657 001
PVDF Western Blotting Membranes	1 roll (30 cm × 3.00 m)	03 010 040 001
Western Blocking Reagent, Solution	100 ml (10 blots, 100 cm ²)	11 921 673 001
	6 × 100 ml (60 blots, 100 cm ²)	11 921 681 001
Bovine Serum Albumin, Fraction V	50 g	10 735 078 001
	100 g	10 735 086 001
	500 g	10 735 094 001
	1 kg	10 735 108 001
Detergents		
Triton X-100	5 × 10 ml	11 332 481 001
Tween 20	5 × 10 ml	11 332 465 001
Nonidet P40	5 × 10 ml	11 332 473 001
Buffers in a Box, Pre-mixed PBS Buffer, 10×	4 l	11 666 789 001

Product	Pack Size	Cat No.
Immunoprecipitation Reagents		
Immunoprecipitation Kit (Protein G)	20 reactions	11 719 386 001
Protein G Agarose	2 ml	11 719 416 001
	5 ml	11 243 233 001
	15 ml	05 015 952 001

^{#)} only available in the U.S.

4.4 Trademarks

COMPLETE, LUMI-LIGHT, BUFFERS IN A BOX, PREMIXED PBS BUFFER, 10× and PHOSSTOP are trademarks of Roche.

Other brands or product names are trademarks of their respective holders.

4.5 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.



Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany