Print

Roche

Anti-c-myc-Peroxidase

Mouse monoclonal antibody (clone 9E10) conjugated to peroxidase

Cat. No. 11 814 150 001 500 µg (500 µl)

Version 06
Content version: July 2009

Store at −15 to −25°C

1. What this Product Does

Contents

The preparation is a frozen liquid containing 500 µl of Anti-c-myc-Peroxidase.

Storage and Stability

Anti-c-myc-Peroxidase is stable until the expiration date printed on the vial when stored at −15 to −25°C. Anti-c-myc-Peroxidase contains bovine serum albumin (5 mg/ml) and 7.5% sucrose as stabilizers and 0.02% thymol as a preservative.

Anti-c-myc-Peroxidase is shipped frozen on dry ice.

Application

Single-step detection of c-myc-tagged recombinant proteins by western blot analysis. Use of Anti-c-myc-Peroxidase eliminates the need for a secondary detection step.

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 crossreact 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(2)
Subtype	Mouse $IgG_1\kappa$.
Purity	Prior to conjugation, the Anti-c-myc monoclonal antibody was ⩾ 90% pure as determined by SDS-PAGE and HPLC analyses.

2. How to Use this Product

2.1 Before You Begin

Working concentration

- Thaw the undiluted Anti-c-myc-Peroxidase, and store on ice prior to use.

Convenient aliquots of the undiluted antibody may be stored at −15 to −25°C.

- For a typical experiment using a 10 cm × 10 cm membrane, dilute 10 µl of undiluted Anti-c-myc-Peroxidase in a total volume of 10 ml Blocking Buffer shortly before use.

2.2 Procedure

Western Blot

The following method has been developed specifically for the Anti-c-myc-Peroxidase. Detection can be performed using a colorimetric (*e.g.*, BM Blue POD Substrate, precipitating*) or fluorescent substrate. For optimum sensitivity of antigen detection, use Anti-c-myc-Peroxidase along with PVDF Western Blotting Membranes* and the Lumi-Light Western Blotting Substrate*.

Step	Action
❶	Perform electrophoresis according to standard protocols (3). Pre-wet a PVDF membrane in 100% methanol, and subsequently equilibrate the PVDF membrane for at least 5 minutes in transfer buffer containing 20% methanol, 24 mM Tris, and 194 mM glycine. Perform western transfer to the PVDF membrane.
❷	Prepare a 1:10 dilution of Western Blocking Reagent* in PBS (phosphate-buffered saline, 1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.0) with 0.1% Tween 20. Ten milliliters of working strength Western Blocking Reagent provides sufficient volume to cover a 10 cm × 10 cm PVDF membrane.
❸	Block the membrane for 1 hour at +15 to +25°C in the working strength Western Blocking Reagent from step 2, agitating gently.
❹	Dilute working strength Western Blocking Reagent (prepared in step 2) 1:1 in PBS containing 0.1% Tween 20. To prepare working strength anti-c-myc-Peroxidase reagent, add 10 µl of anti-c-myc-Peroxidase to 10 ml of this diluted Western Blocking Reagent solution (1:1,000 antibody dilution). Ten milliliters provides sufficient volume to cover a 10 cm × 10 cm PVDF membrane.
❺	Incubate the blocked membrane with the working strength anti-c-myc-Peroxidase for 1 hour at +15 to +25°C with gentle agitation.
❻	Wash the membrane four times, 15 min per wash, with 10 ml PBS, 0.1% Tween 20.
❼	Prepare a Detection Solution according to the protocol described in the Instructions for Use of the Lumi Light Western Blotting Substrate.
❽	Add the Detection Solution prepared in step 8 to the membrane, and incubate the membrane for 1 minute.
❾	Drain excess Detection Solution from the membrane, and wrap the blot in plastic wrap. Expose the membrane to X-ray film (*e.g.*, Lumi-Film Chemiluminescent Detection Film*) in a film cassette for 10 – 60 seconds. For optimal signal strength conditions of substrate development and X-ray film exposure may need adjusting to experimental parameters.

3. Troubleshooting

Problem	Possible Cause	Recommendation
Chemiluminescent or chromogenic signal weak or not visible	Poor isolation of tagged protein	Use a different cell lysis procedure
	Antibody too dilute	Double the concentration of the Anti-c-myc-Peroxidase.
	Too little protein on the gel	Add more protein to gel.
	Poor transfer of proteins from gel to membrane	• Verify efficiency of protein transfer from gel to membrane by silver staining the remaining gel. • To improve transfer efficiency increase the electrical current and/or the transfer time for the blot. • Be sure there are no air bubbles between the membrane and gel during transfer.
	Wrong type of membrane	For maximum signal, use PVDF membranes for transfer.
	Antibody incubation too short	Incubate Anti-c-myc-Peroxidase with the membrane blot for a longer time.
	Signal development time too short	Double the development time.
	Wash time too long or too stringent	• Shorten the washing time. • Omit Tween 20 from the Wash Buffer.
	Enzyme on antibody conjugate inactivated by preservative	Do not use sodium azide in any western blot reagent if you use POD-conjugated antibodies.
	Substrate inactive	Make fresh dilution of substrate or start with a different stock of substrate.
	Epitope tag sequence is not detectable due to: • Proteolytic cleavage • Low level of expression • Premature translation termination resulting in loss of C-terminal tag sequence	• Include protease inhibitors in lysis buffer. • Use alternative expression system or optimize your expression system. • Insert multiple tag sequences into target protein to increase avidity of antibody reaction. • Use alternative insertion site within the target gene for the epitope tag sequence.
High background, additional bands on blot	Antibody too concentrated	Decrease concentration of Anti-c-myc-Peroxidase by half.
	Wash time too short	Increase washing time.
	Incubation of membrane with substrate too long	Leave blot membrane in substrate for a shorter time.
	Wrong type membrane	For minimum background, use PVDF membranes for transfer.
	Blocking Reagent too dilute	Use nonfat dry milk (5% w/v) dissolved in PBST as Blocking Solution and antibody diluent. ⚠ High concentrations of nonfat dry milk may reduce specific signal as well as background.
	Contaminated reagents or equipment	• Use clean equipment, freshly prepared buffers, and new membranes. • Always avoid touching membranes with bare hands; use gloves and forceps.
	Signal development time too long	Reduce development time by half.
	Additional bands	Determine the specificity of the anti-c-myc-Peroxidase: Include a negative-control cell extract prepared from the host organism and lacking the c-myc-tagged protein.

4. 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 (1, 4). However, subsequent studies (1) 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 intracompartmental 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.

Molecular Biology

The DNA sequence, which encodes the c-myc-peptide epitope, can be added to a target gene by oligonucleotide-mediated, site-directed mutagenesis using the polymerase chain reaction (5, 6). The c-myc-peptide epitope sequence has been successfully fused to target proteins at their amino terminal end, carboxy terminal end, or at various sites within the target-protein open reading frame.

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 (2). 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. Anti-c-myc antibody was purified by a protein-G method, then conjugated to horseradish peroxidase. After conjugation, Anti-c-myc-Peroxidase is fractionated and pooled to remove unconjugated anti-c-myc monoclonal antibody.

Quality

To confirm the quality of each new lot, the anti-c-myc-Peroxidase is function-tested by western blot analysis relative to a reference standard. A lysate from a transfected cell line expressing a c-myc-tagged protein is resolved by SDS-PAGE, then transferred to a PVDF membrane. When incubated with the blot membrane at a concentration of 1.0 µg /ml (a dilution of 1:1,000), the Anti-c-myc-Peroxidase specifically binds to the recombinant c-myc-tagged protein. The antigen/antibody-peroxidase complex on the membrane is visualized with a chemiluminescent substrate. A negative control extract is included for comparison.

References

1 Evan, GI, Lewis, GK, Ramsay, G and Bishop, JM (1985) *Mol. Cell. Biol.* **5**, 3610-3616.
2 Nisen, PD, Zimmerman KA, Cotter, SV, Gilbert, F and Alt, FW (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, CC, Varmus, HE and Bishop, M (1983) *Natl. Acad. Sci. USA* **80,** 1707-1711.
5 Cravchik, A. & Matus, A. (1993) Gene 137: 139-143.
6 Kolodziej, PA & Young, RA (1991) *Methods Enzymol.* **194**, 508-519.

Product Citations

- Ditzen C, Jastorff AM, Kessler MS, Bunck M, Teplytska L, Erhardt A, Krömer SA, Varadarajulu J, Targosz B-S, Sayan-Ayata EF, Holsboer F, Landgraf R and Turck CW (2006) Protein Biomarkers in a Mouse Model of Extremes in Trait Anxiety. *Mol. Cell. Proteomics*, **5** 1914 - 1920.
- Mérai Z, Kerényi Z, Kertész S, Magna M, Lakatos L and Silhavy D. (2006): Double-Stranded RNA Binding May Be a General Plant RNA Viral Strategy To Suppress RNA Silencing. *J. Virol.*, **80** 5747 - 5756.
- Cheng Wang, Han Seok Ko, Bobby Thomas, Fai Tsang, Katherine C.M. Chew, Shiam-Peng Tay, Michelle W.L. Ho, Tit-Meng Lim, Tuck-Wah Soong, Olga Pletnikova, Juan Troncoso, Valina L. Dawson, Ted M. Dawson, and Kah-Leong Lim (2005) Stress-induced alterations in parkin solubility promote parkin aggregation and compromise parkin's protective function *Hum. Mol. Genet.*,**14** 3885 - 3897.
- Hyun Sook Chae, Francois Faure, and Joseph J. Kieber (2003) The eto1, eto2, and eto3 Mutations and Cytokinin Treatment Increase Ethylene Biosynthesis in Arabidopsis by Increasing the Stability of ACS Protein. *PLANT CELL*, **15** 545.

5. Supplementary Information

5.1 Conventions

Text Conventions
To make information consistent and easy-to-read, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled A, B, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols
In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations

D	Aspartic acid	K	Lysine
I	Isoleucine	Q	Glutamine
E	Glutamic acid	S	Serine
L	Leucine	A	Alanine
G	Glycine	M	Methionine
N	Asparagine	P	Proline
R	Arginine	T	Threonine
V	Valine	Y	Tyrosine
H	Histidine	C	Cysteine

5.2 Changes to Previous Version

- Editorial changes

5.3 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.

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 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
rGFP	50 µl	11 814 524 001
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 *EASY*packs	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 *EASY*packs	11 836 170 001 04 693 159 001
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* 10 tablets in *EASYpacks*	04 906 837 001 04 906 845 001
Transfection Reagents		
X-tremeGENE 9 DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	06 365 779 001 06 365 787 001 06 365 809 001
X-tremeGENE HP DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	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, (4,000 cm^2 membrane)	12 015 200 001
Lumi-Light^{PLUS} Western Blotting Substrate	100 ml, (1,000 cm^2 membrane)	12 015 196 001

Product	Pack Size	Cat No.
Lumi-Film Chemiluminescent Detection Film	100 films (8 × 10 inches 20.3 x 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) 6 × 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 × 10 ml	11 332 481 001
Tween 20	5 × 10 ml	11 332 465 001
Nonidet P40 Substitute	5 × 10 ml	11 332 473 001
Buffers in a Box, Premixed PBS Buffer, 10×	4 l	11 666 789 001

5.4 Trademarks

5.5 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