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

Anti-c-myc-Peroxidase
from mouse IgG$_1$κ

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Mouse monoclonal antibody (clone 9E10) conjugated to peroxidase.

Cat. No. 11 814 150 001	500 µg 500 µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Anti-c-myc-Peroxidase	Frozen liquid containing bovine serum albumin (BSA) (5 mg/ml) and 7.5% sucrose as stabilizer, and 0.02% thymol as a preservative.	1 vial, 500 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Anti-c-myc-Peroxidase	Store at −15 to −25°C. Once thawed, aliquot and store undiluted antibody at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For western blotting

See section, **Working Solution** *for additional information on preparing solutions.*

- Standard electrophoresis equipment
- 100% methanol
- PVDF Membranes*
- Transfer buffer
- Western Blocking Reagent*
- PBS*
- Tween 20*
- Lumi-Light Western Blotting Substrate*
- X-ray film or Lumi-Film*

1.4. Application

Anti-c-myc-Peroxidase is used for the single-step detection of c-myc-tagged recombinant proteins by western blot analysis.

Using Anti-c-myc-Peroxidase eliminates a secondary detection step.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Epitope tagging

The DNA sequence, which encodes the c-myc-peptide epitope, can be added to a target gene by oligonucleotide-mediated, site-directed mutagenesis using PCR. 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.

Working Solution

Solution	Composition/Preparation	For use in...
Anti-c-myc-Peroxidase working solution	⚠ ***Thaw undiluted Anti-c-myc-Peroxidase and store on ice prior to use.*** 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.	Preparation of antibody working solution.
Transfer buffer	20% methanol, 24 mM Tris, 194 mM glycine	Western blotting
Wash buffer	PBS, 0.1% Tween 20*	Western blotting
Blocking buffer (working strength Western Blocking Reagent)	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. *i* *10 ml of working strength Western Blocking Reagent is sufficient for a 10 cm × 10 cm PVDF membrane.*	Western blotting
Detection solution	See Instructions for Use of the Lumi-Light Western Blotting Substrate*.	Western blotting

2.2. Protocols

Western blotting

The following method has been developed for Anti-c-myc-Peroxidase. Perform detection using a colorimetric 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*.

See section, **Working Solution** *for additional information on preparing solutions.*

1. Perform SDS gel electrophoresis according to standard protocols.

2. Pre-wet a PVDF membrane in 100% methanol.
   – Equilibrate the membrane for at least 5 minutes in Transfer buffer.

3. Perform western blot transfer to the membrane.

4. Block the membrane for 1 hour at +15 to +25°C in the Blocking buffer (working strength Western Blocking Reagent), agitating gently.

5. Dilute working strength Western Blocking Reagent (Blocking buffer) 1:1 in PBS containing 0.1% Tween 20.
   – Add 10 µl of Anti-c-myc-Peroxidase to 10 ml of this diluted Western Blocking Reagent solution (1:1,000 antibody dilution) to prepare the working strength Anti-c-myc-Peroxidase reagent.
   10 ml is sufficient to cover a 10 cm × 10 cm PVDF membrane.

6. Incubate the blocked membrane with the working strength Anti-c-myc-Peroxidase for 1 hour at +15 to +25°C with gentle agitation.

7. Wash the membrane 4 ×, 15 minutes per wash, with 10 ml PBS, 0.1% Tween 20 (Wash buffer).

8. Add the Detection solution to the membrane and incubate for 1 minute.

9. Drain excess Detection solution from the membrane and wrap the blot in plastic wrap.

10. Expose the membrane to X-ray film or Lumi-Film* in a film cassette for 10 to 60 seconds.
    For optimal signal strength, conditions for substrate development and X-ray film exposure may need to be adjusted to experimental parameters.

2.3. Parameters

Epitope

EQKLISEEDL

Purity

≥90% as determined by SDS-PAGE and HPLC (prior to conjugation).

Specificity

Anti-c-myc recognizes the 9E10 epitope (sequence EQKLISEEDL), which was derived from the human c-myc protein. The monoclonal antibody against the c-myc epitope is well characterized 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.

3. Troubleshooting

Observation	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.
		Eliminate 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, or 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 of 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. ⚠ ***Do not touch membranes with bare hands; always use gloves and forceps.***
	Signal development time too long.	Reduce development time by half.
	Additional bands present.	Determine the specificity of the Anti-c-myc-Peroxidase by including a negative control cell extract prepared from the host organism and lacking the c-myc-tagged protein.

4. Additional Information on this Product

4.1. Test Principle

Background Information

Anti-c-myc was originally developed to study c-myc, one of a family of nuclear proteins that were found in several types of human tumors. Subsequent studies used anti-c-myc to detect and purify proteins whose DNA coding sequences were fused to the coding sequence of the c-myc epitope by recombinant DNA techniques. Epitope tagging studies can be used to:

- Determine size, intracellular localization, and abundance of proteins produced by newly discovered genes.
- Track intra-compartmental sorting of a family of proteins.
- Analyze the function of individual protein domains.
- Verify post-translational modification of proteins.
- Monitor fate of transfected proteins.
- Monitor receptor binding and internalization of exogenous proteins.
- Discover the function of proteins that are difficult to purify or share epitopes with a number of other proteins.
- Study 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 to 439 in the human c-myc protein.

① Spleen cells were then fused with SP2/0 myeloma cells to produce the 9E10 hybridoma clone.

② Antibody was produced by cells cultured in a fetal calf serum-supplemented culture medium.

③ Anti-c-myc antibody was purified by a protein-G method, then conjugated to horseradish peroxidase.

④ After conjugation, Anti-c-myc-Peroxidase was fractionated and pooled to remove unconjugated Anti-c-myc monoclonal antibody.

4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Western Blocking Reagent, Solution	100 ml, 10 blots, 100 cm^2	11 921 673 001
	6 x 100 ml, 60 blots, 100 cm^2	11 921 681 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
Lumi-Light Western Blotting Substrate	1 kit, 4,000 cm^2 membrane, 400 blots with 10 x 10 cm	12 015 200 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany