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

Anti-c-myc
from mouse IgG$_1$κ

Version: 07
Content Version: June 2021

Mouse monoclonal antibody (clone 9E10)

Cat. No. 11 667 149 001	200 µg
Cat. No. 11 667 203 001	5 mg 1 ml

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Catalog number	Content
1	Anti-c-myc (clone 9E10)	▪ White lyophilizate in PBS. ▪ 0.2% gelatin for stability.	11 667 149 001	1 vial, 200 µg
		▪ Frozen liquid in PBS, pH 7.4 ▪ 5 mg/ml	11 667 203 001	1 vial, 1 ml

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	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For reconstitution and dilution of Anti-c-myc

- PBS*
- Tris-buffered saline (TBS)
- Tween 20*

For western blotting

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

- SDS gel electrophoresis equipment
- PVDF Membranes*
- Western Blocking Reagent*
- PBS*
- Tween 20*
- Secondary, Peroxidase-conjugated anti-mouse antibody
- Lumi-Light Western Blotting Substrate*
- X-ray film or Lumi-Film Chemiluminescent Detection Film*

1.4. Application

Anti-c-myc (clone 9E10) allows detection of native human c-myc protein and recombinant epitope-tagged proteins that contain the c-myc epitope in numerous applications, such as:

- Immunoblotting, such as western and dot blots
- Immunocytochemistry
- Immunoprecipitation

2. How to Use this Product

2.1. Before you Begin

General Considerations

Epitope tagging

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.
- Insert the c-myc peptide coding sequence into the target gene by oligonucleotide-mediated site-directed mutagenesis.

Working Solution

Solution	Composition/Preparation	For use in...
Anti-c-myc (9E10) stock solution	▪ Add 500 µl PBS* to the lyophilizate to a final concentration of 0.4 mg/ml. ▪ Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***	Anti-c-myc (9E10) working solution
Anti-c-myc (9E10) working solution	Dilute the Anti-c-myc (9E10) stock solution with Dilution buffer to a final concentration of 1 to 10 µg/ml ⓘ *Determine optimal dilution buffer and dilution conditions for each specific application and method.*	Detection
Anti-c-myc (9E10) working solution for western blot	Dilute the Anti-c-myc (9E10) stock solution with Blocking solution B to a final concentration of 1 µg/ml.	Detection
Anti-POD-conjugated antibody	Dilute the secondary, POD-conjugated antibody with Blocking solution B to a final concentration of 1:4,000.	Detection
Transfer buffer	20% methanol, 24 mM Tris base, 194 mM glycine.	Western blot transfer
Washing buffer (PBST)	Dilute Tween 20* to 0.1% (v/v) final concentration in PBS, pH 7.5.	Washing
Dilution buffer	Dilute Tween 20* to 0.1% (v/v) final concentration in TBS.	Anti-c-myc (9E10) working solution and Anti-POD-conjugated antibody
Blocking solution A	1x PBST, containing 10% (w/v) Western Blocking Reagent*.	Blocking
Blocking solution B	1x PBST, containing 5% (w/v) Western Blocking Reagent*.	Anti-c-myc (9E10) working solution for western blot

2.2. Protocols

Western blotting

The following method has been developed for the Anti-c-myc antibody. Optimization for specific experimental systems may be required.

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

1. Lyse cells containing the c-myc epitope and prepare the lysates for SDS gel electrophoresis.
2. Perform electrophoresis on cell lysates.
3. Perform western blot transfer to a PVDF membrane* in transfer buffer.
4. Transfer the membrane to a tray.
   – Incubate for 1 hour at +15 to +25°C or overnight at +2 to +8°C with Blocking solution A.
5. Wash membrane 3 × with Washing buffer.
6. Incubate the blot with Anti-c-myc (9E10) working solution for western blot for 1 to 2 hours at +15 to +25°C with gentle rotation.
7. Wash the membrane 3 × with Washing buffer.
8. Incubate the membrane with the secondary, POD-conjugated antibody for 1 hour at +15 to +25°C with gentle rotation.
9. Wash the membrane 3 × with Washing buffer.
10. Prepare Lumi-Light Western Blotting Substrate* according to the Instructions for Use.
    – Apply Lumi-Light Substrate to the membrane.
11. Expose the membrane to X-ray film or Lumi-Film*.
    – For a 1 minute substrate development, initially perform a 1 to 5 minute film exposure.
    The conditions for development and exposure may vary.

2.3. Parameters

Epitope

EQKLISEEDL

Purity

≥90% as determined by SDS-PAGE and HPLC.

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. Additional Information on this Product

3.1. 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 After purification with Protein G, the antibody was either lyophilized in a 200 µg pack size or stored as a 5 mg liquid pack size.

3.2. Quality Control

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

4. Supplementary Information

4.1. 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.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. 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

4.4. Trademarks

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

4.5. License Disclaimer

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

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

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

4.8. 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.

0621.05035872001 ©

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