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Product datasheet for AM26688AF-N

c-Myc (MYC) Mouse Monoclonal Antibody [Clone ID: PL14]

Product data:

Product Type:	Primary Antibodies
Clone Name:	PL14
Applications:	IF, IP, WB
Recommended Dilution:	Western blot: 1 µg/ml. Immunoprecipitation: 5 µg/ml. Immunocytochemistry: 2 µg/ml. For details see protocols below.
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	GST-6myc-Tag fusion protein
Specificity:	This antibody can be used for epitope-tagging using the amino acid sequence EQKLISEEDL (Myc-Tag).
Formulation:	PBS containing 50% glycerol, pH 7.2, without preservatives State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-A Sepharose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	v-myc avian myelocytomatosis viral oncogene homolog
Database Link:	P01106

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Background: Epitope tagging is a widely accepted technique that fuses an epitope peptide to a protein as a marker for gene expression. With this technique, the gene expression can be easily monitored on Western blotting, immunoprecipitation and immunofluorescence utilizing an antibody that recognizes such an epitope. Amino acid sequences that are widely used for the epitope tagging are as follows; YPYDVPDYA (HA-Tag), EQKLISEEDL (Myc-Tag) and YTDIEMNRLGK (VSV-G-Tag), which correspond to the partial peptide of Influenza hemagglutinin protein, Human c-myc gene product and Vesicular stomatitis virus glycoprotein respectively.

Synonyms: myc tag, myc-tag, c-myc tag

Note: This product was originally produced by MBL International.

Protocol:

SDS PAGE & Western Blotting

1) Boil all samples for 3~5 minutes. Load 10 µl of cell lysate or tissue homogenate (1~10 µg total protein) and electrophoresis in a 1 mm thick SDS-polyacrylamide gel.
2) Transfer to polyvinylidene difluoride (PVDF) membrane at 200mA for 1hour in a semi-dry transfer system. (Transfer Buffer: 25mM Tris, 190mM glycine, 20% MeOH).
3) For reducing non-specific binding, treat the membrane with 5% Skim Milk/ PBS for 1hour at 37°C, or overnight at 4°C.
4) Incubate with primary antibody diluted as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
5)Wash the membrane 3 times with PBS containing 0.05%Tween20 for 5~10 minutes each.
6)Incubate for 45 minutes at room temperature with Horseradish Peroxidase conjugated goat anti-mouse IgG diluted to 1:3000 with PBS.
7) Wash the membrane 3 times with PBS containing 0.05% Tween20 for 10 minutes each.
8) Incubate the membrane with Amersham ECL Reagent for 1 minute. Remove excess ECL Reagent from the membrane by dabbing with a Kimwipe, and seal it in plastic wrap.
9) Expose to ECL hyperfilm in a dark room for 30 seconds. Develop the film as usual for autoradiogram or X-ray. The conditions for exposure and development may vary.

Immunoprecipitation

1) Rinse the cells twice with PBS, then lyse the cells with the addition of 1.2 ml of cold Lysis buffer (50 mM Hepes pH 7.2-7.6, 250 mM NaCl, 0.1% NP40, 10 %Glycerol, 5 mM EDTA), voltex well and put the cells on ice for 30 minutes.
2) Remove the lysate by centrifugation of the cells at 12000 X g for 10 minutes at 4°C.
3) Add the antibody at the amount of as suggested in APPLICATIONS to the supernatant containing approximately 100~500 µg total protein, vortex and incubate with gentle agitation for 30 minutes at 4°C.
4) Add 50 µl of 50 % ProteinA-Sepharose, vortex and incubate with gentle agitation at 4°C for 1 hour.
5) Wash 5 times by centrifugation at 2500 X g for 10 seconds in Lysis buffer.
6) Dissolve the pellet in 20 µl of Laemli SDS sample buffer, boil for 3~5 minutes, and

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centrifuge for 5 minutes. Load 10~20 µl of the supernatant onto the gel and electrophorese normally. Transfer to PVDF membrane and probe with appropriate antibodies.

Immunocytochemical staining

For cultured cell : Immunofluorescence staining Fixing: Rinse the cells on glass slide in PBS and fix cells with 4% paraform aldehyde/PBS (pH 7.5).

Staining:

1) Incubate in primary antibody at the concentration of as suggested in APPLICATIONS diluted with PBS containing 0.1% Triton X 100 for 1 hour at room temperature. (The optimal antibody concentration will depend on several variables and the abundance of the antigen.)

2) Wash the cells 3 times in PBS for 10 minutes each.

3) Incubate in Rhodamine-conjugated anti-mouse IgG (H+L) diluted 1: 100 with PBS for one hour at room temperature.

4) Wash the cells in PBS for 15 minutes.

5) Mounting and microscopic analysis.

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