For Research Use Only.
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MONOCLONAL ANTIBODY

Anti-Myc-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M047-3	PL14	Mouse IgG1	100 µL	2 mg/mL

BACKGROUND: Epitope tagging has widely been accepted technique that fuses an epitope peptide to a certain protein as a marker for gene expression. With this technique, the gene expression can be easily monitored on western blotting, immunoprecipitation and immunofluorescence utilizing with an antibody that recognizes such an epitope. Amino acid sequences that are widely used for the epitope tagging are as follow; YPYDVPDYA (HA-tag), EQKLISEEDL (Myc-tag) and YTDIEMNRLGK (VSV-G-tag), which corresponding to the partial peptide of Influenza hemagglutinin protein, human c-myc gene product and Vesicular stomatitis virus glycoprotein respectively.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone PL14) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with 6myc-tag fusion protein.

FORMULATION: 200 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with Myc-tag peptide sequence on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 5 µg/sample
Immunohistochemistry; Not tested
Immunocytochemistry; 2 µg/mL
Flow cytometry; Not tested
RNP Immunoprecipitation (RIP); Not tested*
Chromatin Immunoprecipitation (ChIP); Not tested*

*It is reported that this antibody can be used in RNP Immunoprecipitation[1), 2)] and Chromatin Immunoprecipitation[3)].

1) Arimoto-Matsuzaki, K., *et al., Nat. Commun.* **7**, 10252 (2016) [RIP]
2) Tani, H., *et al.*, *RNA Biol.* **9**, 1370-1379 (2012) [RIP]
3) Bermejo, R., *et al.*, *Methods Mol. Biol.* **582**, 103-118 (2009) [ChIP]

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
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REFERENCES:
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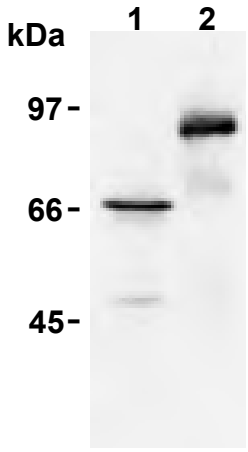

Western blotting analysis of Myc-tag expression in 6Myc/Heparanase transfected 293T cells (1) and 6Myc/cdc25 transfected 293T cells (2) using M047-3.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Wash the 1 x 10^7 cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
3) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.

6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
8) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T (5 minutes x 3).
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into the cell extract. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells for one slide, then incubate in a CO_2 incubator overnight.)
2) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature.
3) Wash the glass slide 3 times with PBS.
4) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
5) Wash the glass slide 3 times with PBS.
6) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
7) Wash the glass slide 3 times with PBS.
8) Add Rhodamine-conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
9) Wash the glass slide 3 times with PBS.
10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.

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