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MONOCLONAL ANTIBODY

Anti-Myc-tag mAb-Agarose

Code No.	Clone	Subclass	Quantity
M047-8	PL14	Mouse IgG1 κ	Gel: 200 µL

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 follows; 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 of anti-Myc-tag monoclonal antibody covalently coupled to 200 µL of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.09% sodium azide) for a total volume of 400 µL.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody recognizes Myc-tag peptide sequence on Immunoprecipitation.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; 20 µL of gel slurry
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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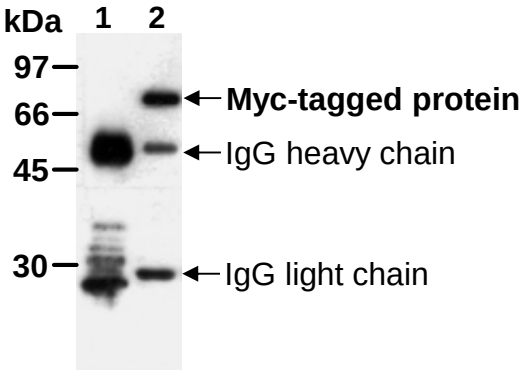
REFERENCES:

1) Shin, C., *et al., Sci. Rep*. **7**, 46097 (2017) [IP]
2) D'Lima, N. G., *et al., Nat. Chem. Biol.* **13**, 174-180 (2017) [IP]
3) Ono, R., *et al., Sci. Rep*. **5**, 18327 (2015) [IP]
4) Kato, K., *et al., Mol. Cell* **53**, 617-630 (2014) [IP]



Immunoprecipitation of Myc-tagged protein from transfectant with mouse IgG1 (1) or M047-8 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with Anti-Myc-tag mAb (MBL, code no. M047-3).*

PROTOCOL:

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volumes 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 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 2-4 times.

8) Resuspend the agarose in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
9) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
10) 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 manufacturer's manual for precise transfer procedure.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
12) Incubate the membrane with 1 μg/mL of Anti-Myc-tag mAb (MBL, code no. M047-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody to be used will depend on the conditions.)
13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
14) 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.
15) Wash the membrane with PBS-T (5 minutes x 6).
16) 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.
17) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

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