For Research Use Only.
Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-His-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M136-3	2D8	Mouse IgG2b	100 µL	1 mg/mL

BACKGROUND: Expression vectors containing a protein and a tag peptide are commonly used. His-tag fusion protein expression system is preferably used in various laboratories, because its simple protein purification step by heavy metal, such as nickel, affinity chromatography. This specific antibody for His-tag fusion protein is useful tool for monitoring of the fusion protein expression and affinity purification.

SOURCE: This antibody was purified from hybridoma (clone 2D8) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with the carrier protein conjugated 6xHis peptide.

FORMULATION: 100 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 His-tagged protein on Immunoprecipitation.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; 0.5-2 µg/sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:
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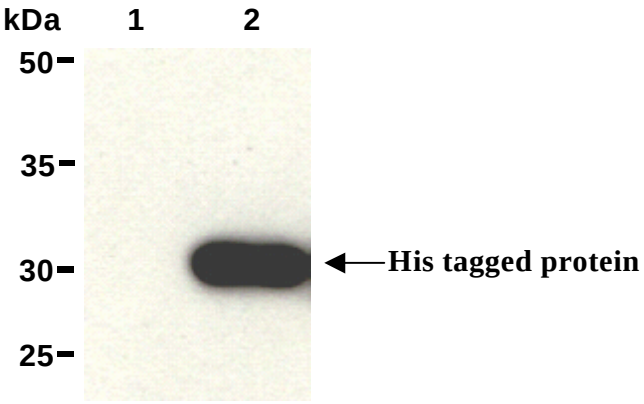
REFERENCES:
1) Sakaguchi, M., *et al*., *PLoS One*. **6**, e23132 (2011) [IP]
2) Lee, J. H., *et al*., *J. Virol*. **80**, 3844-3852 (2006)
3) Muro, S., *et al*., *J. Cell Sci*. **116**, 1599-1609 (2003)
4) Isoyama, T., *et al*., *J. Biol. Chem*. **277**, 39634-39641 (2002)
5) Isoyama, T., *et al*., *J. Biol. Chem*. **276**, 21863-21869 (2001)
6) Toshima, J., *et al*., *Mol. Biol. Cell* **12**, 1131-1145 (2001)



Immunoprecipitation of His-tag from N-terminal His- and DDDDK-tagged protein with Mouse IgG2b (1) or M136-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with Anti-DDDDK-tag (PM020).*

Immunoprecipitation
1) Add primary antibody as suggested in the **APPLICATIONS** into 2 µg of recombinant protein in 200 µL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40]. 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.
2) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
3) Resuspend the agarose with cold Lysis buffer.
4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Repeat steps 2)-4) 2-4 times.
6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
7) Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
8) 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% MeOH). See the manufacture's manual for precise transfer procedure.
9) 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.
10) Incubate the membrane with 1:1,000 of Anti-DDDDK-tag

pAb (MBL; code no. PM020) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)

11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
12) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
13) Wash the membrane with PBS-T (5 minutes x 3 times).
14) 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.
15) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

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http://ruo.mbl.co.jp/