

MONOCLONAL ANTIBODY

# Anti-His-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M136-3	2D8	Mouse IgG2b	100 $\mu$ L	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  $\mu$ g IgG in 100  $\mu$ L 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with His-tagged protein on Immunoprecipitation.

## APPLICATIONS:

Western blotting; Not tested  
Immunoprecipitation; 0.5-2  $\mu$ g/sample  
Immunohistochemistry; Not tested  
Immunocytochemistry; Not tested  
Flow cytometry; Not tested

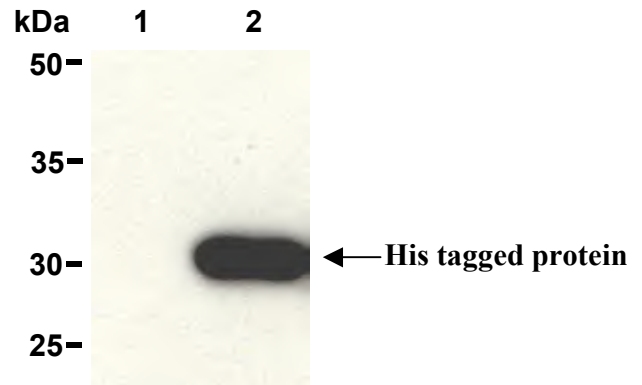
Detailed procedure is provided in the following **PROTOCOL**.

## INTENDED USE:

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

## 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).

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

## PROTOCOLS:

### Immunoprecipitation

- 1) Add primary antibody as suggested in the **APPLICATIONS** into 2  $\mu$ g of recombinant protein in 200  $\mu$ L 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  $4^{\circ}\text{C}$ . Add 20  $\mu$ L of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at  $4^{\circ}\text{C}$ .
- 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  $\mu$ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 7) Load 10  $\mu$ L 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<sup>2</sup> 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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