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

MBL

Anti-His-tag pAb

Code No.	Quantity	Form
PM032MS	20 µL	Affinity Purified

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 rabbit serum using affinity column. The rabbit was immunized with carrier protein (*CP*) conjugated 6xHis synthetic peptide.

FORMULATION: 20 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

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

REACTIVITY: This antibody recognizes His-tagged protein on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1:1,000
Immunoprecipitation; 5 µL/sample
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested*
*It is reported that PM032 can be used for Immunocytochemistry in the reference number 3).
Flow cytometry; Not tested

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

REFERENCES:

1) Lee, Y., *et al.*, *Nat. Plants* **3**, 825-832 (2017) [WB]
2) Nishiyama, A., *et al.*, *Nature* **502**, 249-253 (2013) [WB]
3) Takemoto, M., *et al.*, *Cereb. Cortex* **21**, 1925-1934 (2011) [IC]
4) Kojima, K., *et al.*, *Genes Cells* **14**, 1155-1165 (2009) [IP]
5) Lee, J. H., *et al.*, *J. Virol*. **80**, 3844-3852 (2006)
6) Muro, S., *et al.*, *J. Cell Sci*. **116**, 1599-1609 (2003)
7) Isoyama, T., *et al.*, *J. Biol. Chem*. **277**, 39634-39641 (2002)
8) Isoyama, T., *et al.*, *J. Biol. Chem*. **276**, 21863-21869 (2001)
9) Toshima, J., *et al.*, *Mol. Biol. Cell* **12**, 1131-1145 (2001)

INTENDED USE:

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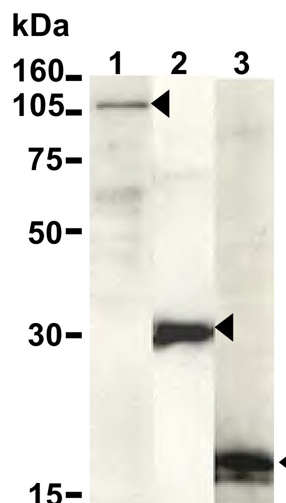

Western blotting analysis of His-tagged protein using PM032.
Lane1: N-terminal of His-tagged protein in 293T
Lane2: N-terminal of His-tagged purified protein
Lane3: C-terminal of His-tagged protein in 293T

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) 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.
3) 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.
4) 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.
5) 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 condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).

7) Incubate the membrane with the 1:10,000 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.
8) Wash the membrane with PBS-T (5 minutes x 6).
9) 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.
10) Expose to an X-ray film in a dark room for 30 seconds. 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 the antibody at the amount of as suggested in the **APPLICATIONS** to the tube. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
6) Resuspend the beads with cold Lysis buffer.
7) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
8) Repeat steps 6)-7) 3-5 times.
9) 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**.)

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