

POLYCLONAL ANTIBODY

Anti-His-tag pAb-Agarose

Code No.

PM032-8

Quantity

Gel: 200 µL

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: 320 µg of anti-His-tag polyclonal 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 is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody recognizes His-tagged protein 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**.

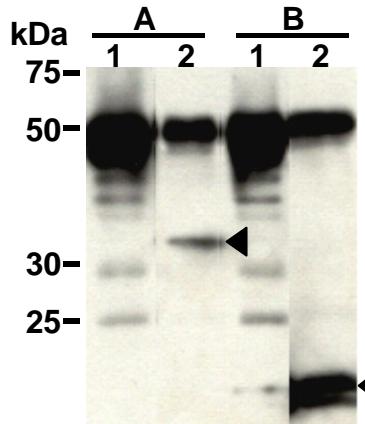
REFERENCES:

- 1) Kajikawa, M., *et al.*, *J. Virol.* **86**, 5288-5296 (2012)
- 2) Shibata, Y., *et al.*, *J. Biochem.* **150**, 679-686 (2011)
- 3) Takemoto, M., *et al.*, *Cereb. Cortex* **21**, 1925-1934 (2011)
- 4) Kojima, K., *et al.*, *Genes Cells* **14**, 1155-1165 (2009)

This antibody is used in these references.

INTENDED USE:

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



Immunoprecipitation of His-tag from N-terminal His-tagged protein (A) and C-terminal His-tagged protein (B) with rabbit IgG (1) and PM032-8 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with PM032.

PROTOCOL:

Immunoprecipitation

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) 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 agarose as suggest in the **APPLICATIONS** into 200 µL of cell extract. Mix well and incubate with gentle agitation for 30-120 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 agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 6) Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
- 7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.

- 8) 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.
- 9) Incubate the membrane with the 1:1,000 anti-His-tag polyclonal antibody (MBL; code no. PM032) diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 11) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 12) Wash the membrane with PBS-T (5 minutes x 3 times).
- 13) 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.
- 14) 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.

RELATED PRODUCTS:

Antibodies

PM032	anti-His-tag (polyclonal)
PM032-8	anti-His-tag-agarose (polyclonal)
D291-3	anti-His-tag (OGHis) (200 µg)
D291-3S	anti-His-tag (OGHis) (50 µg)
D291-7	anti-His-tag HRP-DirecT (OGHis)
D291-8	anti-His-tag-agarose (OGHis)
M089-3	anti-His-tag (6C4)
M136-3	anti-His-tag (2D8)
PM020-8	anti-DDDDK-tag-agarose (polyclonal)
D153-8	anti-GFP-agarose (RQ2)
M165-8	anti-RFP-agarose (3G5)
M047-8	anti-Myc-tag-agarose (PL14)
PM021-8	anti-S-tag-agarose (polyclonal)
PM022-8	anti-T7-tag-agarose (polyclonal)
PM003-8	anti-V5-tag-agarose (polyclonal)
561-8	anti-HA-tag-agarose (polyclonal)
563-8	anti-VSV-G-tag-agarose (polyclonal)

Smart-IP series

3190	Magnetic Rack
D291-9	anti-His-tag-Magnetic beads (OGHis)
M185-9	anti-DDDDK-tag-Magnetic beads (FLA-1)
D153-9	anti-GFP-Magnetic beads (RQ2)
M165-9	anti-RFP-Magnetic beads (3G5)
M132-9	anti-HA-tag-Magnetic beads (5D8)
M180-9	anti-HA-tag-Magnetic beads (TANA2)
M047-9	anti-Myc-tag-Magnetic beads (PL14)
M167-9	anti-V5-tag-Magnetic beads (1H6)
D058-9	anti-Multi Ubiquitin-Magnetic beads (FK2)

Protein Purification Kit

3305	c-Myc-tagged Protein Mild Purification Kit
3305A	c-Myc-tagged Protein Mild Purification Kit (Trial Kit)

3306	c-Myc-tagged Protein Mild Purification Gel with Elution Peptide (1 mL gel, 1 mg peptide)
3307	c-Myc-tagged Protein Mild Purification Gel with Elution Peptide (5 mL gel, 5 mg peptide)
3300-205	c-Myc-tag peptide (5 mg)
3310	His-tagged Protein Purification Kit
3310A	His-tagged Protein Purification Kit (Trial Kit)
3310-205	His-tag peptide (10mg)
3311	His-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 10 mg peptide)
3312	His-tagged Protein Purification Gel with Elution Peptide (5 mL gel, 50 mg peptide)
3315	V5-tagged Protein Purification Kit
3315A	V5-tagged Protein Purification Kit (Trial Kit)
3320	HA-tagged Protein Purification Kit
3320A	HA-tagged Protein Purification Kit (Trial Kit)
3320-205	HA-tag peptide (10 mg)
3321	HA-tagged Protein Purification Gel (1 mL)
3325	DDDDK-tagged Protein Purification Kit
3325A	DDDDK-tagged Protein Purification Kit (Trial Kit)
3325-205	DDDDK-tag peptide (5 mg)
3326	DDDDK-tagged Protein Purification Gel with Elution Peptide (1 mL gel, 5 mg peptide)
3327	DDDDK-tagged Protein Purification Gel with Elution Peptide (5 mL gel, 25 mg peptide)
3328	DDDDK-tagged Protein Purification Gel (5 mL gel)
3329	DDDDK-tagged Protein Purification Gel (25 mL gel)

Other related antibodies and kits are also available.
Please visit our website at <https://ruo.mbl.co.jp/>