

MONOCLONAL ANTIBODY

Anti-His-tag mAb-Agarose

| Code No. | Clone | Subclass | Quantity |
|----------|-------|----------------------|------------------|
| D291-8 | OGHis | Mouse IgG2a κ | Gel: 200 μ L |

BACKGROUND: The His-tag (6xHis-tag) is one of the most common tags used to facilitate the purification of recombinant proteins in *Escherichia coli* or other expression systems.

This product is useful tool for immunoprecipitation of the His-tagged proteins, and it recognizes His-tags placed at N-terminal, C-terminal, and internal regions of the recombinant proteins.

SOURCE: This antibody was purified from hybridoma (clone OGHis) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP-1 with Balb/c mouse splenocyte immunized with 6xHis tagged protein.

FORMULATION: 200 μ g of anti-His-tag monoclonal antibody covalently coupled to 200 μ L of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.1% ProClin 150) for a total volume of 400 μ L.

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

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

APPLICATION:

Immunoprecipitation: 20 μ L of gel slurry/300 μ L of cell extract from 1 x 10⁶ cells

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

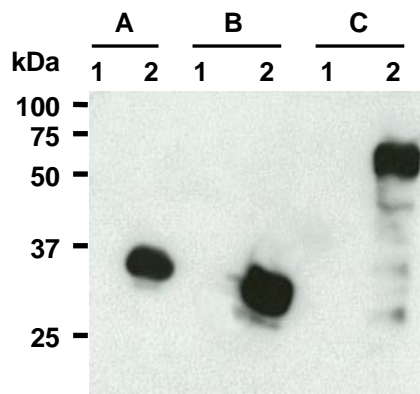
INTENDED USE:

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

REFERENCES:

- 1) Kawada, J., *et al.*, *Int. J. Cancer* **130**, 584-592 (2012)
- 2) Ueyama, T., *et al.*, *J. Biol. Chem.* **286**, 40693-40705 (2011)
- 3) Suzuki, T., *et al.*, *Biochem. Biophys. Res. Commun.* **409**, 70-74 (2011)
- 4) Hiragami-Hamada, K., *et al.*, *Mol. Cell Biol.* **31**, 1186-1200 (2011)

Clone OGHis is used in these references.



Immunoprecipitation of His-tag from N-terminal His-tagged protein (A), Internal His-tagged protein (B), C-terminal His-tagged protein (C) with agarose conjugated mouse IgG1 isotype control, M075-8 (1) or D291-8 (2). After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with D291-7.

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 gel as suggested in the **APPLICATIONS** into 300 μ L of the supernatant. 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 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
- 6) Load 5 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out 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 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.

- 9) Incubate the membrane with 1:5,000 of Anti-His-tag mAb-HRP-DirecT (MBL; code no. D291-7) diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody to be used will be depended on the condition.)
- 10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 11) 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.
- 12) 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.

RELATED PRODUCTS:

Antibodies

| | |
|----------|---|
| D291-3 | Anti-His-tag mAb (OGHis) (200 µL) |
| D291-6 | Anti-His-tag mAb-Biotin (OGHis) |
| D291-7 | Anti-His-tag mAb-HRP-DirecT (OGHis) |
| D291-8 | Anti-His-tag mAb-Agarose (OGHis) |
| D291-A48 | Anti-His-tag mAb-Alexa Fluor® 488 (OGHis) |
| D291-A59 | Anti-His-tag mAb-Alexa Fluor® 594 (OGHis) |
| D291-A64 | Anti-His-tag mAb-Alexa Fluor® 647 (OGHis) |
| M089-3 | Anti-His-tag mAb (6C4) |
| M136-3 | Anti-His-tag mAb (2D8) |
| PM032 | Anti-His-tag pAb (polyclonal) |
| PM032-8 | Anti-His-tag pAb-Agarose (polyclonal) |
| PM020-8 | Anti-DDDDK-tag pAb-Agarose (polyclonal) |
| D153-8 | Anti-GFP mAb-Agarose (RQ2) |
| M165-8 | Anti-RFP mAb-Agarose (3G5) |
| M047-8 | Anti-Myc-tag mAb-Agarose (PL14) |
| PM021-8 | Anti-S-tag pAb-Agarose (polyclonal) |
| PM022-8 | Anti-T7-tag pAb-Agarose (polyclonal) |
| PM003-8 | Anti-V5-tag pAb-Agarose (polyclonal) |
| 561-8 | Anti-HA-tag pAb-Agarose (polyclonal) |
| 563-8 | Anti-VSV-G-tag pAb-Agarose (polyclonal) |

Smart-IP series

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|---------|---|
| 3190 | Magnetic Rack |
| M180-11 | Anti-HA-tag mAb-Magnetic Beads (TANA2) |
| M132-11 | Anti-HA-tag mAb-Magnetic Beads (5D8) |
| M185-11 | Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1) |
| M047-11 | Anti-Myc-tag mAb-Magnetic Beads (PL14) |
| D291-11 | Anti-His-tag mAb-Magnetic Beads (OGHis) |
| D153-11 | Anti-GFP mAb-Magnetic Beads (RQ2) |
| M165-11 | Anti-RFP mAb-Magnetic Beads (3G5) |
| M198-9 | Anti-E-tag mAb-Magnetic beads (21D11) |
| M167-11 | Anti-V5-tag mAb-Magnetic Beads (1H6) |
| D058-9 | Anti-Multi Ubiquitin mAb-Magnetic beads (FK2) |
| M075-11 | Mouse IgG1 (isotype control)-Magnetic Beads (2E12) |
| M076-11 | Mouse IgG2a (isotype control)-Magnetic Beads (6H3) |
| M077-11 | Mouse IgG2b (isotype control)-Magnetic Beads (3D12) |
| M081-11 | Rat IgG2a (isotype control)-Magnetic Beads (2H3) |
| M180-10 | Anti-HA-tag mAb-Magnetic Agarose (TANA2) |
| M132-10 | Anti-HA-tag mAb-Magnetic Agarose (5D8) |
| M185-10 | Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1) |
| M047-10 | Anti-Myc-tag mAb-Magnetic Agarose (PL14) |

| | |
|---------|---|
| D291-10 | Anti-His-tag mAb-Magnetic Agarose (OGHis) |
| D153-10 | Anti-GFP mAb-Magnetic Agarose (RQ2) |
| M165-10 | Anti-RFP mAb-Magnetic Agarose (3G5) |
| M167-10 | Anti-V5-tag mAb-Magnetic Agarose (1H6) |
| M198-10 | Anti-E-tag mAb-Magnetic Agarose (21D11) |

Protein Purification Kits

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|----------|--|
| 3305 | c-Myc-tagged Protein Mild Purification Kit |
| 3305A | c-Myc-tagged Protein Mild Purification Kit (Trial Kit) |
| 3340 | c-Myc-tagged Protein Magnetic Purification Kit |
| 3340A | c-Myc-tagged Protein Magnetic 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) |
| 3306K | c-Myc-tagged Protein Purification Cartridge (1 mL x 1) |
| 3310 | His-tagged Protein Purification Kit |
| 3310A | His-tagged Protein Purification Kit (Trial Kit) |
| 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) |
| 3310-205 | His-tag peptide (10 mg) |
| 3317 | V5-tagged Protein Purification Kit Ver.2 |
| 3317A | V5-tagged Protein Purification Kit Ver.2 (Trial Kit) |
| 3341 | V5-tagged Protein Magnetic Purification Kit |
| 3341A | V5-tagged Protein Magnetic Purification Kit (Trial Kit) |
| 3318 | V5-tagged Protein Purification Gel Ver.2 |
| 3315-205 | V5-tag peptide (10 mg) |
| 3320 | HA-tagged Protein Purification Kit |
| 3320A | HA-tagged Protein Purification Kit (Trial Kit) |
| 3342 | HA-tagged Protein Magnetic Purification Kit |
| 3342A | HA-tagged Protein Magnetic Purification Kit (Trial Kit) |
| 3321 | HA-tagged Protein Purification Gel (1 mL) |
| 3320-205 | HA-tag peptide (10 mg) |
| 3325 | DDDDK-tagged Protein Purification Kit |
| 3325A | DDDDK-tagged Protein Purification Kit (Trial Kit) |
| 3343 | DDDDK-tagged Protein Magnetic Purification Kit |
| 3343A | DDDDK-tagged Protein Magnetic Purification Kit (Trial Kit) |
| 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) |
| 3325-205 | DDDDK-tag peptide (5 mg) |
| 3326K | DDDDK-tagged Protein Purification Cartridge (1 mL x 1) |

Other related antibodies and kits are also available.

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