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Not for use in diagnostic procedures.**

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MONOCLONAL ANTIBODY

Anti-His-tag mAb-Agarose

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

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 µg of anti-His-tag monoclonal antibody covalently coupled to 200 µL 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 µL.

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 µL of gel slurry/300 µL of cell extract from 1 x 10^6 cells

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

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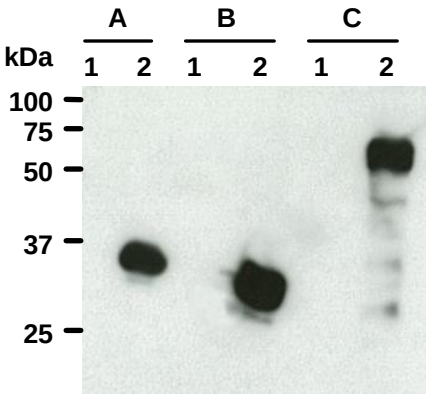
REFERENCES:
1) Kawada, J., *et al., Int. J. Cancer* **130**, 584-592 (2012)
2) Ueyama, T., *et al., J. Biol. Chem.* **286**, 40693-40705 (2011)
3) Suzuki, T., *et al., Biochem. Biophys. Res. Commun.* **409**, 70-74 (2011)
4) Hiragami-Hamada, K., *et al., Mol. Cell Biol.* **31**, 1186-1200 (2011)

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.*

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

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 µL 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 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
6) Load 5 µL 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/cm^2 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.

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