

MONOCLONAL ANTIBODY

Anti-His-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M089-3	6C4	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: A variety of plasmids contain DNA that encodes an N-terminal tag consisting of six histidine (His) residues, followed by an extended multiple cloning sites. The His-tag fusion protein expression system is commonly used because the 6xHis-tag on the recombinant proteins allows for efficient coupling to Ni⁺⁺ affinity resins and purification by a single step chromatography. This specific antibody for His-tag fusion protein is useful for monitoring of the fusion protein expression and affinity purification.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 6C4) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with synthetic peptide 6xHis.

FORMULATION: 100 µg IgG in 100 µL 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°C.

REACTIVITY: This antibody reacts with His-tag on Western blotting.

APPLICATIONS:

Western blotting: 1 µg/mL for chemiluminescence detection system

Immunoprecipitation: Not recommended

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested

Flow cytometry: Not tested

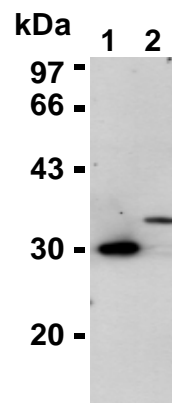
Detailed procedure is provided in the following **PROTOCOL.**

INTENDED USE:

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

REFERENCES:

- 1) Ma, L., *et al.*, *Dis. Markers* **35**, 317-325 (2013) [WB]
- 2) Hayashi, T., *et al.*, *Cell Host Microbe*. **12**, 20-33 (2012) [WB]
- 3) Higashimoto, Y., *et al.*, *J. Clin. Microbiol.* **50**, 1245-1251 (2012) [WB]
- 4) Porath, J., *et al.*, *Protein Express. Purif.* **3**, 263-281 (1992)



Western blot analysis of His-Azami-Green (1) and His-EGFP (2) using M089-3.

PROTOCOL:

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/cm² 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.
- 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 the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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 times).
- 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 5 minutes.

Develop the film as usual. The condition for exposure and development may vary.

RELATED PRODUCTS

Antibodies

M048-3 Anti-GFP mAb (1E4)
D153-3 Anti-GFP mAb (RQ2)
D153-6 Anti-GFP mAb-Biotin (RQ2)
D153-8 Anti-GFP mAb-Agarose (RQ2)
598 Anti-GFP pAb (polyclonal)
598-7 Anti-GFP pAb-HRP-Direct (polyclonal)
PM073 Anti-Renilla GFP pAb (polyclonal)
M155-3 Anti-RFP mAb (8D6)
M165-3 Anti-RFP mAb (3G5)
M165-8 Anti-RFP mAb-Agarose (3G5)
M204-3 Anti-RFP mAb (1G9)
M204-7 Anti-RFP mAb-HRP-Direct (1G9)
M208-3 Anti-RFP mAb Cocktail (1G9, 3G5)
PM005 Anti-RFP pAb (polyclonal)
PM005-7 Anti-RFP pAb-HRP-Direct (polyclonal)
M180-3 Anti-HA-tag mAb (TANA2) (200 µL)
M180-6 Anti-HA-tag mAb-Biotin (TANA2)
M180-7 Anti-HA-tag mAb-HRP-Direct (TANA2)
561 Anti-HA-tag pAb (polyclonal) (100 µL)
561-7 Anti-HA-tag pAb-HRP-Direct (polyclonal)
561-8 Anti-HA-tag pAb-Agarose (polyclonal)
M132-3 Anti-HA-tag mAb (5D8)
M185-3L Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-7 Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
PM020 Anti-DDDDK-tag pAb (polyclonal)
PM020-7 Anti-DDDDK-tag pAb-HRP-Direct (polyclonal)
PM020-8 Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3 Anti-Myc-tag mAb (My3) (200 µL)
M192-6 Anti-Myc-tag mAb-Biotin (My3)
M047-3 Anti-Myc-tag mAb (PL14)
M047-7 Anti-Myc-tag mAb-HRP-Direct (PL14)
M047-8 Anti-Myc-tag mAb-Agarose (PL14)
562 Anti-Myc-tag pAb (polyclonal) (100 µL)
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)
M167-3 Anti-V5-tag mAb (1H6)
PM003 Anti-V5-tag pAb (polyclonal)
PM003-7 Anti-V5-tag pAb-HRP-Direct (polyclonal)
PM003-8 Anti-V5-tag pAb-Agarose (polyclonal)
PM021 Anti-S-tag pAb (polyclonal)
PM070 Anti-E-tag pAb (polyclonal)
PM022 Anti-T7-tag pAb (polyclonal)
563 Anti-VSV-G-tag pAb (polyclonal)
M071-3 Anti-GST-tag mAb (3B2)
PM022 Anti-GST-tag pAb (polyclonal)

M095-3 Anti-Luciferase mAb (2D4)
PM016 Anti-Luciferase pAb (polyclonal)
PM047 Anti-Renilla Luciferase pAb (polyclonal)
M094-3 Anti-β-galactosidase mAb (5A3)
M203-3 Anti-β-galactosidase mAb (6F4)
PM049 Anti-β-galactosidase pAb (polyclonal)
M091-3 Anti-MBP (Maltose Binding Protein) mAb (1G12)
M013-3 Anti-Thioredoxin (Trx-tag) mAb (2C9)
PM015 Anti-CBD (Chitin Binding Domain) pAb (polyclonal)
PM071 Anti-Calmodulin Binding Protein-tag pAb (polyclonal)
M211-3 Anti-Strep-tag II mAb (4F1)
M214-3 Anti-mini-AID-tag mAb (1E4)

Smart-IP series

3190 Magnetic Rack
D153-11 Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11 Anti-RFP mAb-Magnetic Beads (3G5)
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)
M198-9 Anti-E-tag mAb-Magnetic beads (21D11)
M167-11 Anti-V5-tag mAb-Magnetic Beads (1H6)
D058-11 Anti-Multi Ubiquitin mAb-Magnetic Beads (FK2)
M075-11 Mouse IgG1 (isotype control)-Magnetic Beads
M076-11 Mouse IgG2a (isotype control)-Magnetic Beads
M077-11 Mouse IgG2b (isotype control)-Magnetic Beads
M081-11 Rat IgG2a (isotype control)-Magnetic Beads
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

3320 HA-tagged Protein PURIFICATION KIT
3321 HA-tagged Protein PURIFICATION GEL (1 mL)
3320-205 HA-tag peptide (2 mg x 5)
3325 DDDDK-tagged Protein PURIFICATION KIT
3326 DDDDK-tagged Protein PURIFICATION GEL (Gel: 1 mL x 1, Peptide: 1 mg x 5)
3325-205 DDDDK-tag peptide (1 mg x 5)
3305 c-Myc-tagged Protein MILD PURIFICATION KIT Ver. 2
3306 c-Myc-tagged Protein MILD PURIFICATION GEL (Gel: 1 mL x 1, Peptide: 1 mg x 5)
3310 His-tagged Protein PURIFICATION KIT
3311 His-tagged Protein PURIFICATION GEL (Gel: 1 mL x 1, Peptide: 2 mg x 5)
3317 V5-tagged Protein PURIFICATION KIT Ver.2
3318 V5-tagged Protein PURIFICATION GEL Ver.2 (1 mL)

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