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MBL
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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
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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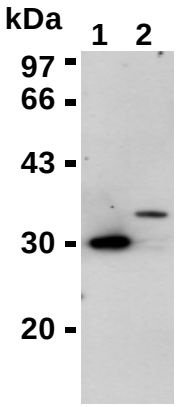
REFERENCES:

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

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

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/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 the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with 1:10,000 of Anti-IgG (H+L chain) (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).
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.

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