

MONOCLONAL ANTIBODY

Anti-His-Tag

Code No.	Clone	Subclass	Quantity	Concentration
M089-3	6C4	Mouse IgG1	100 µg	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 6 x His-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 hybridoma (clone 6C4) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with synthetic peptide 6 His.

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

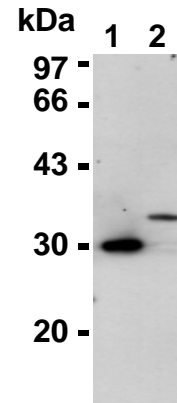
Detailed procedure is provided in the following **PROTOCOL**.

REFERENCE:

1) Porath, J., *et al.*, *Protein Express. Purif.* **3**, 263-281 (1992)

INTENDED USE:

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



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 PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (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:

- 598 Anti-GFP (polyclonal)
- M048-3 Anti-GFP (1E4)
- D153-3 Anti-GFP (RQ2)
- D153-8 Agarose conjugated anti-GFP (RQ2)
- PM005 Anti-RFP (polyclonal)
- M136-3 Anti-His-tag (2D8)
- PM002 Anti-His-Tag (polyclonal)
- 561 Anti-HA-Tag (polyclonal) (0.1 mL)
- 561-5 Anti-HA-Tag (polyclonal) (0.5 mL)
- 561-8 Agarose conjugated anti-HA-Tag (polyclonal) (0.5 mL)
- M132-3 Anti-HA-tag (5D8)
- 562 Anti-Myc-Tag (polyclonal) (0.1 mL)
- 562-5 Anti-Myc-Tag (polyclonal) (0.5 mL)
- M047-3 Anti-Myc-Tag (PL14)
- M047-6 Biotin labeled anti-Myc-Tag (PL14)
- M047-7 HRP conjugated anti-Myc-Tag (PL14)
- M047-8 Agarose conjugated anti-Myc-Tag (PL14)
- PM020 Anti-DDDDK-Tag (polyclonal)
- PM020-8 Agarose conjugated anti-DDDDK-Tag (polyclonal)
- PM003 Anti-V5-Tag (polyclonal)
- PM003-8 Agarose conjugated anti-V5-Tag (polyclonal)
- 563 Anti-VSV-G-Tag (polyclonal) (0.1 mL)
- 563-5 Anti-VSV-G-Tag (polyclonal) (0.5 mL)
- 563-8 Agarose conjugated anti-VSV-G-Tag (polyclonal)
- M071-3 Anti-GST-Tag (3B2)
- M071-7 HRP conjugated anti-GST-Tag (3B2)
- PM013 Anti-GST-Tag (polyclonal)
- M013-3 Anti-Thioredoxin (2C9)
- PM021 Anti-S-Tag (polyclonal)
- PM021-8 Agarose conjugated anti-S-Tag (polyclonal)
- PM022 Anti-T7-Tag (polyclonal)
- PM022-8 Agarose conjugated anti-T7-Tag (polyclonal)
- M091-3 Anti-MBP (1G12)
- M094-3 Anti- β -galactosidase (5A3)
- M095-3 Anti-Luciferase (2D4)
- PM016 Anti-Luciferase (polyclonal)
- PM015 Anti-Chitin Binding Domain (polyclonal)
- M102-3 Anti-Azami-Green (2F11)
- M103-3 Anti-Azami-Green (3D10)
- PM011 Anti-Azami-Green (polyclonal)
- M117-3 Anti-Dronpa-Green (4D12)
- M118-3 Anti-Dronpa-Green (2F6)
- M106-3 Anti-Kaede (2F4)
- M125-3 Anti-Kaede (3B1)
- PM012 Anti-Kaede (polyclonal)
- M126-3 Anti-Keima-Red (2F7)
- M127-3 Anti-Keima-Red (3C9)
- M128-3 Anti-Kikume Green-Red (5B3)
- M129-3 Anti-Kikume Green-Red (2D3)
- M104-3 Anti-Kusabira-Orange (1H7)
- M105-3 Anti-Kusabira-Orange (2G9)
- M116-3 Anti-Midoriishi-Cyan (2C1)
- M130-3 Anti-Midoriishi-Cyan (5B7)

- CY-M1027 Anti-Adenovirus Hexon (AF-3H12)
- CY-M1026 Anti-Baculovirus envelope gp64 (AF-2C8)
- 3300 c-Myc tagged Protein MILD PURIFICATION KIT
- 3300A c-Myc tagged Protein MILD PURIFICATION KIT (Trial Kit)
- 3306 c-Myc tagged Protein MILD PURIFICATION GEL (1mL)
- 3307 c-Myc tagged Protein MILD PURIFICATION GEL (5mL)