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

MONOCLONAL ANTIBODY

Anti-GST-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M071-3	3B2	Mouse IgG2b κ	100 µL	1 mg/mL

BACKGROUND: Expression vectors containing a protein and a tag protein are commonly used. Bacterial glutathione S-transferase (GST)-tag fusion protein expression system is preferably used in various laboratories because of its simple protein purification step by an affinity chromatography. This specific antibody for GST-tag fusion protein is useful tool for monitoring of the fusion protein expression and affinity purification.

SOURCE: This antibody was purified from hybridoma (clone 3B2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Balb/c mouse splenocyte immunized with recombinant bacterial glutathione S-transferase.

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 recombinant GST-tag on Western blotting.

APPLICATIONS:
Western blotting; 1 µg/mL
Immunoprecipitation; Not recommended*
*MBL code no. PM013 is suitable for Immunoprecipitation.
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

REFERENCES:
1) Wang, W., *et al., Nucleic Acids Res.* **40**, 981-995 (2012)
2) Nakagawa, T., *et al., Genes Cells.* **12**, 709-719 (2007)
3) Yoshida, M., *et al., J Dermatol Sci.* **41**, 21-30 (2006)
4) Toji, S., *et al., Genes Cells.* **9**, 383-397 (2004)
5) Yabuta, N., *et al., Genes Cells.* **8**, 413-421 (2003)
6) Shimazaki, N., *et al., Genes Cells.* **7**, 639-651 (2002)

Clone 3B2 is used in these references.

INTENDED USE:
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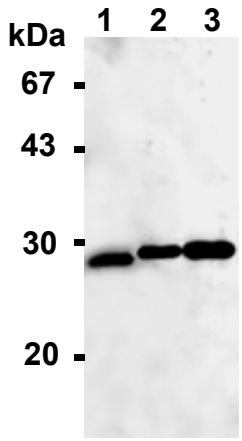

Western blot analysis of GST expression in pGEX3X (1), pGEX4T-1 (2) and pGEX5X-1 (3) using M071-3.

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

PROTOCOLS:
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% 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 to be used 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 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 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

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