

MONOCLONAL ANTIBODY

# Anti-GST-tag mAb

Code No.	Clone	Subclass	Quantity	Concentration
M071-3	3B2	Mouse IgG2b $\kappa$	100 $\mu$ L	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  $\mu$ g IgG in 100  $\mu$ L 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  $\mu$ g/mL for chemiluminescence detection system

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**.

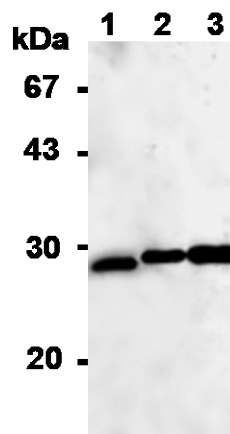
## REFERENCES:

- 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:

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



**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  $\mu$ L 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<sup>2</sup> 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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