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

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POLYCLONAL ANTIBODY

Anti-GST-tag pAb

Code No.	Quantity	Form
PM013	100 µL	Rabbit IgG

BACKGROUND: Bacterial Glutathione S-transferase (GST) from *Schistosoma japonicum* is a widely used protein fusion partner, since it provides exhibits little effect on the biological function of the protein of interest. The GST-tag fusion protein expression system allows for efficient coupling to glutathione (GSH) agarose affinity resins and purification by a single step chromatography. This specific antibody for GST-tag fusion protein is useful for monitoring of the fusion protein expression and affinity purification. It does not recognize mammalian GST.

SOURCE: This antibody was purified from rabbit serum using protein A agarose. The rabbit was immunized with recombinant bacterial glutathione S-transferase, which was purified from pGEX-5X-3 vector using GSH column.

FORMULATION: 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 GST on Western blotting.

APPLICATIONS:
Western blotting; 1:1,000
Immunoprecipitation; 5 µL
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:
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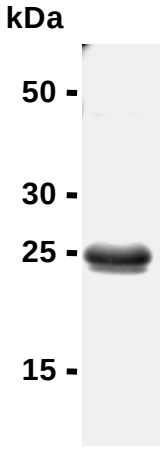
REFERENCES:
1) Izumi, H., and Kaneko, Y., *Cancer Res.* **74**, 5620-5630 (2014) [WB]
2) Sato, M., *et al.*, *Int. Immunol.* **23**, 493-502 (2011) [WB]
3) Zhou, J. and Blissard, G. W., *J. Virol.* **82**, 4449-4460 (2008) [WB]
4) Yamaguchi, T., *et al., J. Cell Biol.* **171**, 431-436 (2005)
5) Yamamoto, H., *et al., Mol. Cell Biol.* **19**, 3829-3841 (1999)

This antibody is used in these references.

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



Western blot analysis of GST expression in pGEX-4T-1 using PM013.

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 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 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) 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.

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add the antibody at the amount of as suggest in **APPLICATIONS** to the supernatant containing approximately 100~500 µg total protein. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Add 20 µL of 50% protein A agarose beads resuspended in the Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
5) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
   (See **SDS-PAGE & Western blotting**.)

RELATED PRODUCTS:

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