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

Anti-GST-tag mAb

CODE No.	M209-3
CLONALITY	Monoclonal
CLONE	GT5
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	Recombinant GST protein
FORMULATION	PBS containing 50% glycerol. No preservative is contained.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

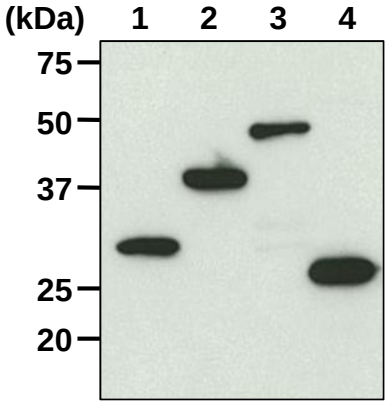
Western blotting	1 µg/mL
Immunoprecipitation	2.5 µg/sample

For more information, please visit our web site **https://ruo.mbl.co.jp/**.

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

SDS-PAGE & Western blotting

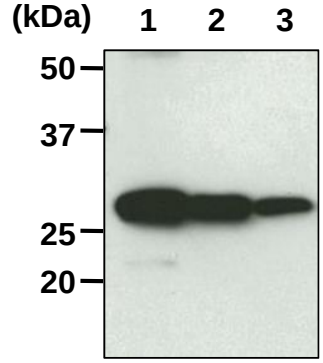
1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the sample for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3).
8) Incubate the membrane with the 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3).
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.



Western blotting analysis of GST-tagged protein

Lane 1: Myc-tagged GST/BL21 DE3 *E.coli* lysate, 50 ng
Lane 2: GST-hLC3 (pGEX-4T-1), 20 ng
Lane 3: GST-tagged protein X (pGEX-6P-1), 20 ng
Lane 4: GST (pGEX-6P-3), 20 ng

Immunoblotted with Anti-GST-tag mAb (MBL, code no. M209-3)



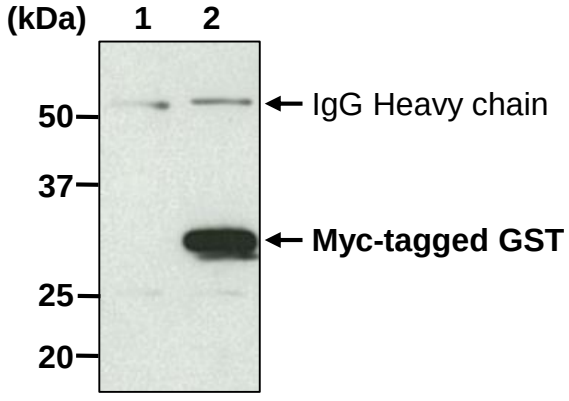
Western blotting analysis of GST protein

Lane 1: HEK293T cell lysate from 1x10^4 cells + GST protein 20 ng
Lane 2: HEK293T cell lysate from 1x10^4 cells + GST protein 8 ng
Lane 3: HEK293T cell lysate from 1x10^4 cells + GST protein 0.4 ng

Immunoblotted with Anti-GST-tag mAb (MBL, code no. M209-3)

Immunoprecipitation

1) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 400 µL of IP buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 30 min. at room temperature.
2) Wash the beads once with 1 mL of IP buffer.
3) Add 5 µg of recombinant Myc-tagged GST protein and 400 µL of IP buffer. Incubate with gentle agitation for 1 hr. at 4°C.
4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with 1 mL of IP buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 3 times.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
9) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacturer's manual for precise transfer procedure.
11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
13) Incubate the membrane with 1 µg/mL of Anti-GST-tag mAb (MBL, code no. M209-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
14) Wash the membrane with PBS-T (5 min. x 3).
15) Incubate the membrane with the 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
16) Wash the membrane with PBS-T (5 min. x 3).
17) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
18) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
19) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of GST protein

Sample: Myc-tagged GST protein
Lane 1: Mouse IgG1 (isotype control) (MBL, code no. M075-3)
Lane 2: Anti-GST-tag mAb (MBL, code no. M209-3)

Immunoblotted with Anti-GST-tag mAb (MBL, code no. M209-3)