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

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POLYCLONAL ANTIBODY

Anti-GST-P pAb

Code No.	Quantity	Form
311	100 µL	Purified IgG

BACKGROUND: Placental glutathione S-transferase (GST-P), a member of glutathione S-transferase, is known for its specific expression during rat hepatocarcinogenesis and has been used as a reliable tumor marker for experimental rat hepatocarcinogenesis.

SOURCE: This antibody was purified from rabbit serum using protein A agarose. The rabbit was immunized with purified rat liver glutathione S-transferase P.

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 glutathione S-transferase P (27 kDa) on Western blotting.

APPLICATIONS:
Western blotting; 1:1,000-1:2,000
Immunoprecipitation; Not tested
Immunohistochemistry; 1:1,000-1:2,000
This antibody can be used for staining of frozen sections and paraffin sections.
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:
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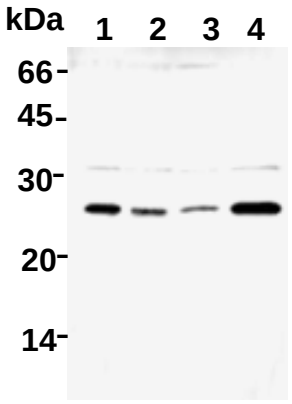
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells and Tissue	Jurkat	WR19L, kidney	PC12
Reactivity on WB	+	+	+

RELATED PRODUCTS:
Please visit our website at https://ruo.mbl.co.jp/.

REFERENCES:
1) Hokaiwado, N., *et al., Carcinogenesis* **29**, 1134-1138 (2008) [WB]
2) Takahashi, M., *et al., Carcinogenesis* **29**, 2218-2226 (2008) [IHC]
3) Suzuki, R., *et al., Carcinogenesis* 27, 619-630 (2006) [IHC]
4) Ueno, S., *et al., Clin. Cancer Res.* **11**, 5645-5650 (2005) [IHC]
5) Hasumura, M., *et al., Toxicol. Sci.* **86**, 61-67 (2005) [IHC]
6) Sukata, T., *et al., Am. J. Pathol.* **165**,1479-1488 (2004) [IHC]
7) Suzuki, S., *et al., Carcinogenesis* **25**, 439-443 (2004) [IHC]
8) Nakaji, M., *et al., Carcinogenesis* **25**, 389-397 (2004) [IHC]
9) Oyama, K., *et al., Carcinogenesis* **23**, 885-892 (2002) [IHC]
10) Denda, A., *et al., Carcinogenesis* **23**, 245-256 (2002) [IHC]
11) Sato, K., *et al., PNAS* **82**, 3964-3968 (1985)

As this antibody is widely used, many researches have been reported. These references are a part of such reports.



Western blotting analysis of GST-P expression in mouse kidney (1), WR19L (2), PC12 (3) and Jurkat (4) using 311.

PROTOCOLS:
SDS-PAGE & Western blotting
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).

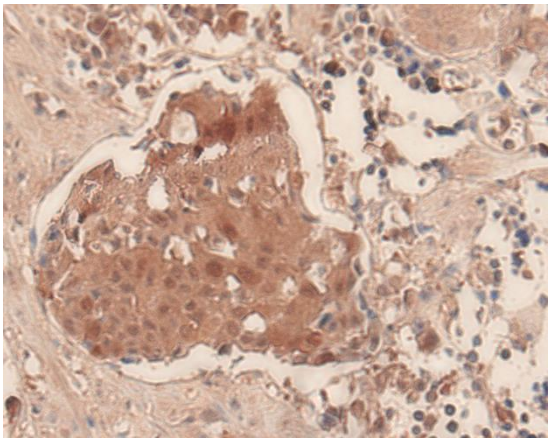
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
5) 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% methanol). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with 5% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 minutes x 3).
9) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3).
11) 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.
12) 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.

(Positive controls for Western blotting; mouse kidney, WR19L, PC12, Jurkat)

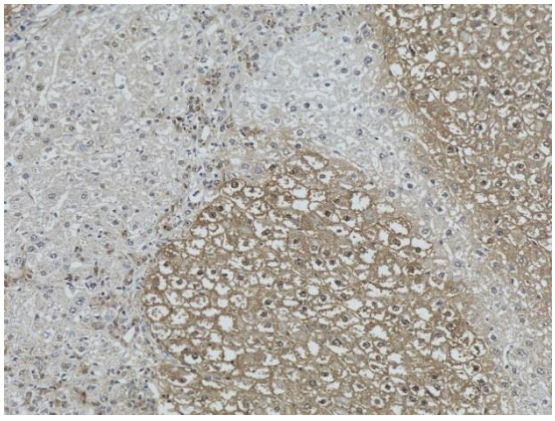
Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS, wipe gently around each section and cover tissues with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Blocking buffer (20 mM HEPES, 1% BSA, 135 mM NaCl, pH 7.2) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the Blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Histostar™ (Ms + Rb) (MBL, code no. 8460). Incubate for 10 minutes at room temperature.
10) Wash the slides twice in PBS for 5 minutes each.
11) Visualize by reacting for 1 minute with Histostar™ DAB (MBL, code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 3 minutes, and then wash the slides 3 times in water for 5 minutes each.
14) Dehydrate by immersing in Ethanol 4 times for 3 minutes each, followed by immersing in Xylene twice for 3 minutes each.
14) Now ready for mounting.

(Positive controls for Immunohistochemistry; rat liver, human stomach)



Immunohistochemical detection of GST-P on human stomach paraffin-embedded section with 311.



Immunohistochemical detection of GST-P in paraffin-embedded section of precancerous rat liver with 311.