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

POLYCLONAL ANTIBODY

Anti-GST-π pAb

Code No.	Quantity	Form
312	100 µL	Purified IgG

BACKGROUND: GST-π is a 26 kDa protein that belongs to the GST superfamily of enzymes which have an important role in detoxification. They catalyze the conjugation of many hydrophobic and electrophophilic compounds with reduced glutathione. There are four main classes: α, µ, π and τ. Results indicate that GST- π is the most accurate marker enzyme for detection of initiated cells during liver carcinogenesis, preneoplasia, colonic carcinoma and lung carcinoma.

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

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- π, and doesn't react with other isozymes on Western blotting.

APPLICATIONS:
Western blotting; 1:500-1:1,000
Immunoprecipitation; Not tested
Immunohistochemistry; 1:500-1:1,000
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat	NIH/3T3	Rat1, PC12	CHO
Reactivity on WB	+	+	+	+

INTENDED USE:
For research use only. Not for clinical diagnosis.

REFERENCES:
1) Tsuchida, S., *et al*., *Cancer Res*. **49**, 5225-5229 (1989)
2) Sato, K., *et al., Adv. Cancer Res.* **52**, 205-255 (1989)
3) Eimoto, H., *et al*., *Carcinogenesis* **9**, 2325-2327 (1988)
4) Shiratori, Y., *et al*., *Cancer Res*. **47**, 6806-6809 (1987)
5) Batist, G., *et al*., *J. Biol. Chem.* **261**, 15544-15549 (1986)
6) Soma, Y., *et al*., *Biochim. Biophys. Acta*. **869**, 247-258 (1986)

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

PROTOCOLS:
SDS-PAGE & Western blotting
1) Wash the cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 3 minutes 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 hour 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 minutes x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% 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.)
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) 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 hr. at room temperature.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) 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.
11) 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; Rat1, PC12, Jurkat, NIH/3T3, and CHO)

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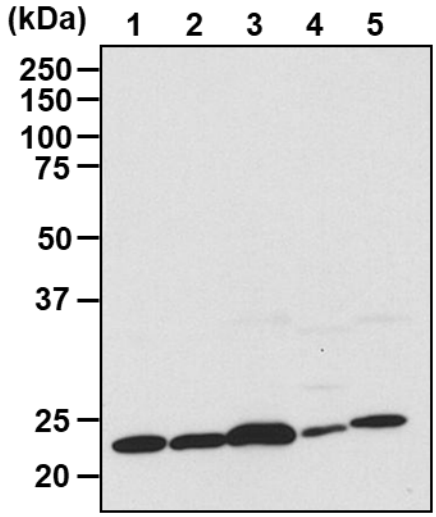
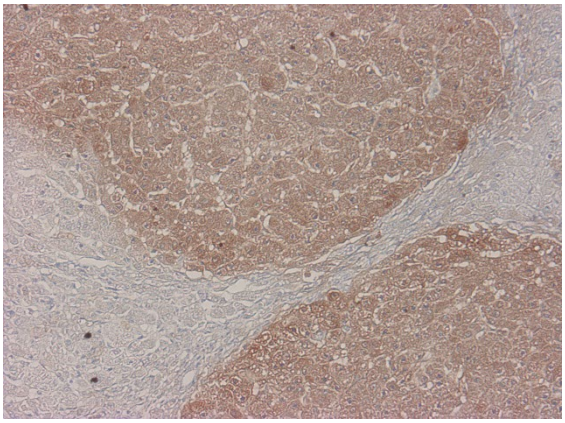


Western blotting analysis of GST-π expression in Rat1(1), PC12 (2), Jurkat (3), NIH/3T3 (4) and CHO (5) using 312.

Immunohistochemical staining for paraffin-embedded sections

1) Deparaffinize the sections with Xylene 3 times for 5 minutes each.
2) Wash the slides with Ethanol 3 times for 5 minutes each.
3) Wash the slides with PBS 3 times for 5 minutes each.
4) Remove the slides from PBS and cover each section with 3% H_2O_2 in PBS for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 2 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) for 5 minutes at room temperature (20~25°C) 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**. Incubate the sections for 1 hour at room temperature.
7) Wash the slides 3 times in PBS for 5 minutes each.
8) Wipe gently around each section and cover tissues with Histostar (Ms + Rb) (MBL; code no. 8460). Incubate for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Visualize by reacting for 3 minutes with Histostar DAB (MBL; code no. 8469) at room temperature. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
11) Wash the slides in water for 5 minutes.
12) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
13) Now ready for mounting.

(Positive control for Immunohistochemistry; Precancerous rat liver)



Immunohistochemical detection of GST-π in paraffin-embedded section of precancerous rat liver with 312.

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