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

POLYCLONAL ANTIBODY

Anti-GST-π

Code No.	Quantity	Form
312	100 µL	IgG fraction

BACKGROUND: GST-π is a 26kDa 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 - 1:1000 for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; 1:500 - 1:1000
Immunocytochemistry; Not tested
Flow Cytometry; Not tested

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

INTENDED USE:

For research use only. Not for clinical diagnosis.

RELATED PRODUCTS:

311 Anti-GST-P (Polyclonal)
311H Anti-GST-P (Polyclonal)

REFERENCES:

1) Boku, N., *et al*., *Jpn. J. Clin. Oncol*. **37**, 275-281 (2007)
2) Gu, M., *et al*., *Cancer Res*. **67**, 3483-3491 (2007)
3) Nagashima, F., *et al*., *Jpn. J. Clin. Oncol*. **35**, 714-719 (2005)
4) Tsuchida, S., *et al*., *Cancer Res*. **49**, 5225-5229 (1989)
5) Sato, K., *et al*., *Adv. Cancer Res*. **52**, 205-255 (1989)
6) Eimoto, H., *et al*., *Carcinogenesis* **9**, 2325-2327 (1988)
7) Shiratori, Y., *et al*., *Cancer Res*. **47**, 6806-6809 (1987)
8) Batist, G., *et al*., *J. Biol. Chem*. **261**, 15544-15549 (1986)
9) Soma, Y., *et al*., *Biochim. Biophys. Acta*. **869**, 247-258 (1986)

This antibody is used in the reference number 1)-3).

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 2 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% MeOH). See the manufacturer's manual for precise transfer procedure.
6) 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.
7) 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.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458)

diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS (5 minutes x 6 times).
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 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

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 and cover each section 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 Protein Blocking Agent (Ultratech HRP Kit; IMMUNOTECH, code no. IM-2391) for 5 minutes to block non-specific antibody 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 suggest 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 Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8.
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8.
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 μL of 30% H_2O_2 in 150 mL PBS. *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 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.
14) Now ready for mounting.