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

MONOCLONAL ANTIBODY

# Anti-Glutathione Peroxidase (Human) mAb

| Code No. | Clone | Subclass | Quantity | Concentration |
|---|---|---|---|---|
| M015-3 | GPX-347 | Mouse IgG1 κ | 100 µL | 1 mg/mL |

**BACKGROUND:** Glutathione peroxidase (GPX) is a selenoprotein which catalyzes the reduction of a variety of hydroperoxides, including lipid peroxide and hydrogen peroxide, thereby protecting biomembrane and essential cellular components against oxidative damage.

**SOURCE:** This antibody was purified from hybridoma (clone GPX-347) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with human erythrocyte glutathione peroxidase.

**FORMULATION:** 100 µg IgG in 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 N-terminal epitope of human GPX (22.5 kDa) on Western blotting.

**APPLICATINS:**

Western blotting; 10 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not recommended
Immunocytochemistry; 1-10 µg/mL
Flow cytometry; Not tested

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

**SPECIES CROSS REACTIVITY:**

| Species | Human | Mouse | Rat |
|---|---|---|---|
| Cells | PC9, HL-60, Jurkat, U937, HeLa, NB4, HepG2, Raji | Liver, kidney, plasma, NIH/3T3 | PC12 |
| Reactivity on WB | + | - | - |

**INTENDED USE:**

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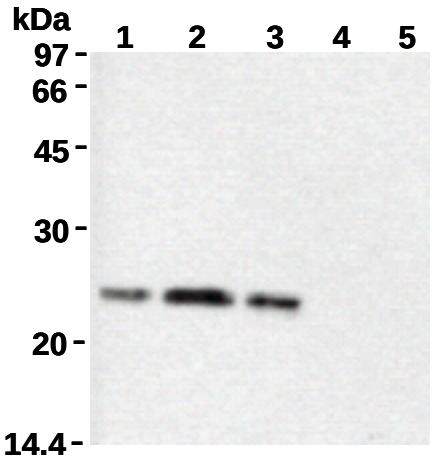
**REFERENCES:**

1) Handy, D.E., *et al., J. Biol. Chem.* **281**, 3382-3388 (2006)
2) Handy, D.E., *et al., J. Biol. Chem.* **280**, 15518-15525 (2005)
3) Perwez Hussain, S., *et al., Cancer Res.* **64**, 2350-2356 (2004)
4) Yin, L., *et al., J. Biol. Chem.* **278**, 35458-35464 (2003)
5) Suemizu H., *et al*., *HYBRIDOMA* **11**, 795-801 (1992)

Clone GPX-347 is used in these references.




***Western blot analysis of GPX expression in Jurkat (1), Raji (2), HeLa (3), NIH/3T3 (4) and PC12 (5) using M015-3.***

**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 sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 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 the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (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.

(Positive controls for Western blotting; Jurkat, Raji and HeLa)

**Immunocytochemistry**

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread $1x10^4$cells for one slide, then incubate in a $CO_2$ incubator for one night.)
2) Fixing: a) or b)
   a) The cells were fixed with methanol at -20°C for 2 minutes and with acetone at 4°C for 5 minutes.
   b) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature. The glass slide was washed with PBS 3 times. Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
3) The glass slide was washed with PBS 3 times.
4) Cover the cells with 0.2% BSA in PBS for 10 minutes to minimize non-specific adsorption of the antibodies to the glass slide.
5) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature (Optimization of antibody concentration or incubation condition is recommended if necessary.)
6) The glass slide was washed with PBS 3 times.
7) Add FITC-conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
8) The glass slide was washed with PBS 3 times.
9) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.