

POLYCLONAL ANTIBODY

Anti-IgG (H+L chain) (Rabbit) pAb-HRP

Code No.	Quantity	Form
458	1 mL	Goat Fab'

SOURCE: This product was produced by the following method.

- (1) The specific antibody was purified using ion exchange chromatography from goat serum immunized with the rabbit IgG (H+L chain).
- (2) After absorbed the reactivity to human IgG using affinity chromatography, the IgG fraction was digested with Pepsin in order to produce the F(ab)'₂.
- (3) After the F(ab)'₂ was reduced, the resulting Fab' was jointed to HRP (horse-radish peroxidase) by the maleimide-ester.
- (4) Subsequently this product was purified from them using gel chromatography.

FORMULATION: 1 mL volume of phosphate buffer containing 1% BSA and preservative.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with rabbit IgG (H+L chain).

APPLICATIONS:

Western blotting; 1:5,000-1:10,000

ELISA; 1:5,000-10,000

Immunoprecipitation; Not tested

Immunohistochemistry; Not recommended

Immunocytochemistry; Not tested

Flow cytometry; Not tested

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

INTENDED USE:

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

NOTE: Use of sodium azide as a preservative substantially inhibits the enzyme activity of horseradish peroxidase.

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

PROTOCOLS:

SDS-PAGE & Western blotting

- 1) Wash cells (approximately 1×10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 20 μ L of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 7) Incubate the membrane with secondary antibody as suggested in the **APPLICATIONS** diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (10 minutes x 3).
- 9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
- 10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
- 11) Expose the membrane onto an X-ray film in a dark room for 10 minutes.
- 12) Develop the film under usual settings. The conditions for exposure and development may vary.

ELISA

- 1) Dilute antigen in coating buffer (0.1 M phosphate buffer).
- 2) Add 100 μ L to each well. Cover the plate and incubate overnight at 4°C.
- 3) Wash the plate 4 times with PBS containing 0.05% Tween-20 (PBS-T).
- 4) Block with blocking buffer (1% BSA, 5% Sucrose, 0.1% NaN₃ in PBS) at 37°C for 1 hour. Wash as in step 3).
- 5) Distribute 100 μ L/well of the capture antibody to each well. Incubate at 37°C for 1 hour. Wash as in step 3).
- 6) Distribute 100 μ L/well of the 1:5,000-10,000 Anti-IgG (H+L chain) (Rabbit) pAb-HRP (MBL, code no. 458) polyclonal antibody to each well.

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- 7) Incubate at room temperature for 1 hour. Wash as in step 3).
- 8) Distribute 100 μL /well of the substrate solution (tetramethyl benzidine solution).
- 9) Incubate at room temperature for 30 minutes.
- 10) Distribute 100 μL /well of 2 M H_2SO_4 to each well and stop enzyme reaction.
- 11) After gentle mixing, determine the absorbance at 450 nm of each well by a spectrophotometer.

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