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

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

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

Code No.	Quantity	Form
330	1 mL	Goat Fab'

SOURCE: This product was produced by the following method.

(1) The specific antibody was purified using ion exchange chromatography from sera of goat was immunized with the mouse 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 mouse IgG (H+L chain) and IgM (H+L chain). This antibody cross reacts with rat.

APPLICATIONS:
Western blotting; 1:5,000-1:10,000
ELISA; 1:5,000-1:10,000
Immunoprecipitation; Not tested
Immunohistochemistry; Not recommended
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:
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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 x 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 µL 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/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 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 µL 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% NaN_3 in PBS) at 37 °C for 1 hour. Wash as in step 3).
5) Distribute 100 µL/well of the capture antibody to each well. Incubate at 37 °C for 1 hour. Wash as in step 3).

6) Distribute 100 μL/well of the 1:5,000-10,000 HRP-conjugated Anti-IgG (H+L chain) (Mouse) pAb-HRP（MBL; code no. 330) polyclonal antibody to each well.
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 50 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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