

POLYCLONAL ANTIBODY

# Anti-IgY (Chicken) pAb-HRP

Code No.  
PM010-7

Quantity  
500 µL

**SOURCE:** This antibody is rabbit polyclonal antibody raised against the purified chicken IgY. The rabbit IgG is affinity purified by chicken IgY coupled agarose beads and the cross reactivity to serum protein of human is absorbed by column chromatography. Fab' fragment of the rabbit IgG is conjugated with horseradish peroxidase (HRP) by maleimide method.

**FORMULATION:** HRP conjugated purified IgG in 0.1 M NaPB/0.1% Proclin150 /0.1 M NaCl /1% BSA (pH 6.5).

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

**REACTIVITY:** This antibody reacts with chicken IgY and chicken IgG. The cross reactivity to IgG of human, mouse, rat, guinea pig, goat and bovine is lower than 1%.

**APPLICATIONS:**

Western blotting: 1:5,000 for chemiluminescence detection system.

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

**SPECIES CROSS REACTIVITY:**

Species	Chicken	Human, Mouse, Rat, Guinea pig, Goat, Bovine
Reactivity on ELISA	+	-

**INTENDED USE:**

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

**RELATED PRODUCTS:**

PM009-7	Anti-IgG (H+L Chain) (Mouse) pAb-HRP
210	Anti-IgA (α chain) (Human) pAb-HRP
546	Anti-IgG (H+L chain) (Goat) pAb-HRP
330	Anti-IgG (H+L chain) (Mouse) pAb-HRP
458	Anti-IgG (H+L chain) (Rabbit) pAb-HRP
206	Anti-IgG (H+L chain) (Human) pAb-HRP
208	Anti-IgG (γ chain) pAb-HRP
212	Anti-IgM (μ chain) (Human) pAb-HRP

**PROTOCOL:**

**SDS-PAGE & Western Blotting**

- 1) Wash the  $1 \times 10^7$  cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
- 2) 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.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture'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 PBS, pH 7.2 containing 1% skimmed milk 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 7) Incubate the membrane with the 1:5,000 Anti-IgY (Chicken) pAb-HRP (MBL; code no. PM010-7) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 3 times).
- 9) 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.
- 10) Expose to an X-ray film in a dark room for 10 minutes. Develop the film as usual. The condition for exposure and development may vary.