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Goat Anti-Rabbit IgG H&L (HRP polymer) Ready-to-Use Kit

Cat. No.: PV-1023

Application: Immunohistochemical (IHC) Staining

Host: Goat

Reactivity: Rabbit

Isotype: IgG

Conjugation: HRP polymer

Size: 3ml / 6ml / 50ml

Storage: Stable for 12 months at 2-8°C. Protect from light.

General Information

Component	Size
Goat anti-rabbit secondary antibody, HRP polymer conjugated	6ml / 50ml
Endogenous peroxidase blocking solution	6ml / 50ml
Normal goat serum	6ml / 50ml

Principle

The detection system facilitates the identification of rabbit IgG antibody bond to an antigen in tissue sections. The specific antibody is pinpointed by a secondary antibody that is conjugated with a horseradish peroxidase (HRP) polymer, designed to specifically recognize rabbit immunoglobulins. The polymer attached complex is subsequently made visible under light microscope using HRP-compatible chromogens, such as diaminobenzidine (DAB).

The provided HRP polymer conjugated secondary antibody significantly address the limitations commonly experienced with traditional immunohistochemistry (IHC) methods, such as poor or inconsistent antigen staining when identifying low-abundance antigens or in situations of suboptimal antibody-antigen binding. By utilizing an HRP polymer conjugate, the sensitivity of detection is dramatically increased and the process is simplified. Moreover, the HRP polymer-based amplification method reduces the amount of primary antibody needed and shortens the secondary antibodies' incubation period.

Protocol

- **1.** Prepare your assay samples and complete antigen retrieval as per your IHC protocol.
- 2. Discard any residual liquid around the tissue section and add 50-100μl of endogenous peroxidase blocking solution, then incubate in a humidity chamber at room temperature (RT) for 15-20 minutes.
- 3. Wash the slides three times with PBS (PH 7.4) for 3 minutes each.
- **4.** Add 50-100μl of normal goat serum to block non-specific binding sites by the incubation for 15-20 minutes in a humidity chamber at RT.
- **5.** Discard normal goat serum (it's not necessary to wash) and apply primary antibody for incubation according to manufacturer's recommended protocol.
- 6. Wash slides 3 times with PBS (PH 7.4) for 5 minutes each.
- 7. Remove remaining liquid around the tissue section and add $50-100\mu l$ of Polymer-HRP goat anti-rabbit secondary antibody, followed by a 15-20 minute incubation at RT in a humidity chamber.
- 8. Wash slides 3 times with PBS (PH 7.4) for 5 minutes each.

- 9. Apply HRP-compatible chromogens to tissue according to manufacturer's recommended instructions.
- **10.** Counterstain and coverslip with a mounting media for microscopy.

Notes

- 1. Please thoroughly read this instruction manual before starting the experiment.
- 2. Please note that any uncareful maneuvers within the IHC assay may impact the final results.
- **3.** Negative results from an IHC staining only indicate that the target antigen was not detected. It would be inappropriate to conclude that the antigen of interest is absent in the samples tested.
- **4.** Be vigilant to avoid skin and eyes contact with the reagent. Should any contact occur, immediately rinse the affected area with plenty of water.
- **5.** The HRP-polymer system can be used in IHC autostainers.