Technical Manual

Anti-Rabbit/Mouse IgG Poly-HRP DAB Two Step IHC Detection System

- Catalogue Code: AGEL3545
- Size: 3 mL / 6 mL / 18 mL / 50 mL
- Research Use Only

1. Product Description

Anti-Rabbit/Mouse IgG Poly-HRP DAB Two Step IHC Detection System utilizes polymerized monovalent Fab fragments of secondary antibodies conjugated with enzymes, replacing both the secondary and tertiary antibodies used in conventional methods. This configuration allows for direct amplification of the antigen-antibody binding signal.

The system maintains the high specificity of antibody-antigen interactions while effectively minimizing steric hindrance caused by large polymer molecules. Compared to the traditional streptavidin-biotin (SP) three-step method, 2-Step Plus offers a simplified, faster protocol with high sensitivity. By eliminating the use of biotin, the system prevents background staining from endogenous biotin. It is suitable for immunohistochemical applications using monoclonal or polyclonal primary antibodies derived from rabbit or mouse. Additionally, the kit includes a polymer-based enhancer designed to improve the detection of bound primary antibodies in macromolecular complexes. A diluent for the concentrated DAB chromogen is also provided to stabilize the working solution and eliminate variability due to water pH, ensuring consistent and reliable color development.

2. Kit components

No	Item	3 mL	6 mL	18 mL	50 mL	Storage
1	3% H_2O_2	3 mL	6 mL	18 mL	50 mL	2~8°C
2	Normal Goat Blocking Buffer (Ready-to-Use)	3 mL	6 mL	18 mL	50 mL	2~8°C
3	Polymer Helper (Ready-to-Use)	3 mL	6 mL	18 mL	50 mL	2~8°C
4	Polyperoxidase-anti-Mouse/Rabbit IgG (Ready-to-Use)	3 mL	6 mL	18 mL	50 mL	2~8°C
5	DAB Concentrate (20×)	150 µL	300 µL	900 µL	2.5 mL	2~8°C
6	DAB Substrate	3 mL	6 mL	18 mL	50 mL	2~8°C
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3. Storage and expiry date

- **Storage Conditions:** Store at 2~8°C, protect from direct light.
- **Shelf-Life:** Valid for 12 months. The reagents are valid within 6 months after opening.
- **Expiration Date:** The expiration date of the kit is clearly indicated on the **outer packaging box**. Do not use the kit beyond this date.

4. Sample dyeing

1. **Deparaffinization and Rehydration:** Deparaffinize and rehydrate paraffin-embedded tissue sections using standard xylene and graded ethanol procedures.
2. **Antigen Retrieval:** Perform antigen retrieval based on the specific requirements of the primary antibody being used.
3. **Blocking Endogenous Peroxidase Activity:** Incubate sections with 3% H_2O_2 for 10 minutes to quench endogenous peroxidase activity.
4. **Washing:** Rinse sections with PBS or TBS buffer for 2 minutes, repeating three times.
5. **Blocking Non-Specific Binding:** Gently blot excess buffer with absorbent paper. Apply Normal Goat Blocking Buffer to the section and incubate at 37°C for 30 minutes.
6. **Primary Antibody Application:** Blot excess blocking buffer and use an oil-based pen to draw a hydrophobic barrier around the tissue section. Apply the appropriately diluted primary antibody and incubate at room temperature or 37°C for 1–2 hours, or overnight at 4°C followed by rewarming at 37°C for 30 minutes. Wash with PBS or TBS for 2 minutes × 3 times.
7. **Polymer Enhancer Application:** Apply Polymer Helper to the section and incubate at room temperature or 37°C for 20 minutes. Wash with PBS or TBS for 2 minutes × 3 times.
8. **Polymer HRP Conjugate Application:** Add Polyperoxidase-anti-Mouse/Rabbit IgG to the tissue and incubate at room temperature or 37°C for 20–30 minutes. Wash with PBS or TBS for 2 minutes × 3 times.
9. **Preparation of DAB Working Solution:** Mix 1 drop (~50 µL) of DAB Concentrate into 1 mL of DAB Substrate to prepare the DAB Working Solution. Mix thoroughly. Prepare fresh before use, protect from light, and use within 4 hours. Discard any unused solution.
10. **Chromogenic Development:** Apply the freshly prepared DAB Working Solution to the tissue. Observe under a microscope; positive staining appears brownish-yellow or brown. If staining is intense or the development time exceeds 10 minutes, rinse the section with tap water to terminate the reaction. Avoid over-staining.
11. **Final Processing:** Rinse the section with deionized water to terminate the chromogenic reaction. Proceed with counterstaining, dehydration, clearing, and mounting as per standard IHC procedure.

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If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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