

## 2-step Plus Poly-HRP Anti Rabbit/Mouse IgG Detection System

Cat. No: E-IR-R211

Size: 3 mL/ 6 mL/ 18 mL

Cat	product	3 mL	6 mL	18 mL	Storage
E-IR-R211A	3% H <sub>2</sub> O <sub>2</sub>	3 mL	6 mL	18 mL	2~8°C
E-IR-R211B	Polymer Helper (Ready-to-Use)	3 mL	6 mL	18 mL	2~8°C
E-IR-R211C	Polyperoxidase-anti-Mouse/Rabbit IgG (Ready-to-Use)	3 mL	6 mL	18 mL	2~8°C
<b>Manual</b>		<b>One Copy</b>			

### Introduction

2-step plus is a two-step immunohistochemical broad spectrum detection reagent. It polymerizes monovalent Fab fragments of secondary antibody and enzyme, which replaces the secondary antibody and tertiary antibody in traditional method, can directly amplify the binding signal of antibody-antigen. This method not only retains the specific binding ability of antibody with antigen, but also can effectively avoid space steric hindrance caused by excessive polymer molecules. Compared with the traditional SP three-step method, this kit has the characteristics of simple, rapid, high-sensitivity. This system abandons the using of biotin, so it can avoid background staining by endogenous biotin. It can be used in IHC, in which the primary antibody is monoclonal/polyclonal antibody derived from rabbit/mouse. The unique polymer auxiliary agent for macromolecular detection system can detect the binding primary antibody better.

### Sample dyeing

1. Dewax and hydrate the paraffin section.
2. Repair antigen of the tissue section according to special requirements of applied primary antibody.
3. Incubate with E-IR-R211A (3% H<sub>2</sub>O<sub>2</sub>) for 10 min to eliminate endogenous peroxidase activity.
4. Wash with PBS or TBS, 2 min×3 times.
5. Add blocking solution (normal goat serum), incubate at 37°C for 30 minutes to block, then discard the excess liquid.
6. Add primary antibody with proper dilution ratio, incubate at 20~37°C for 1~2h or at 4°C overnight (then rewarm at 37°C for 30 min). Wash with PBS or TBS, 2 min×3 times.
7. Add E-IR-R211B (Polymer Helper), incubate at room temperature or 37°C for 20 min. Wash with PBS or TBS, 2 min×3 times.
8. Add E-IR-R211C (Polyperoxidase-anti-Mouse/Rabbit IgG), incubate at room temperature or 37°C for 20~30 min. Wash with PBS or TBS, 2 min×3 times.
9. Color development with DAB.
10. Wash with deionized water to make the section counterstained, dewatered, transparent, then seal the section.

### Storage

Store at 2~8°C, shading light. Avoid of freezing. Valid for 12 months. The reagents are valid within 6 months after opening.