2-step Plus Poly-HRP Anti Mouse/Rabbit IgG Detection System（with DAB solution）

Cat. No: E-IR-R217 **Size: 3 mL/ 6 mL/ 18 mL/ 50 mL**

Cat	product	3 mL	6 mL	18 mL	50 mL	Storage
E-IR-R217A	Normal Goat Blocking Buffer (Ready-to-Use)	3 mL	6 mL	18 mL	50 mL	2~8°C
E-IR-R217B	Polyperoxidase-anti-Mouse/Rabbit IgG (Ready-to-Use)	3 mL	6 mL	18 mL	50 mL	2~8°C
E-IR-R217C	3% H_2O_2	3 mL	6 mL	18 mL	50 mL	2~8°C
E-IR-R217D	DAB Concentrate (25×)	120 µL	240 µL	720 µL	2 mL	2~8°C
E-IR-R217E	DAB Substrate	3 mL	6 mL	18 mL	50 mL	2~8°C
Manual			**One Copy**			

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 or polyclonal antibody derived from mouse or rabbit.

Diluent for DABconcentrated solutionhas been provided in this kit to avoid the influence of the different water acidity and alkalinity on the DAB Chromogenic Agent.

Sample dyeing

1. Dewax and hydrate the paraffin section.
2. Make thermal repair or digestion treatment to antigen of the tissue section if necessary according to antigen/antibody situation.
3. Incubate with E-IR-R217C (3% H_2O_2) for 10 min to eliminate endogenous peroxidase activity. Wash with PBS or TBS, 2 min×3 times.
4. Add E-IR-R217A (Normal Goat Blocking Buffer (Ready-to-Use)), incubate at 37°C for 30 min. Shake off any excess liquid.
5. Add primary antibody (From Mouse or Rabbit) with proper dilution ratio, incubate at 20~37°C for 1~2hor at 4°Covernight (then rewarm at 37°C for 30 min). Wash with PBS or TBS, 2 min×3 times. Dry the section with absorbent paper.
6. Add E-IR-R217B (Polyperoxidase-anti-Mouse/Rabbit IgG), incubate at room temperature or 37°C for 20 min. Wash with PBS or TBS, 2 min×3 times.
7. Add 1 drop (approximately 40 µL) of E-IR-R217D (DAB Concentrate) into each 1 mL of E-IR-R217E (DAB Substrate), mix fully and the mixed reagent is the DAB Working Solution. Prepare fresh solution before use and the prepared solution should be stored in the dark. Fresh prepared DAB Working Solution is valid within 4 hours and the unused solution must be abandoned
8. Add freshly prepared DAB staining solution to each slice, observe under a microscope, and the positive signal is brownish yellow or brown. If the color is obvious or the color development time exceeds 10 minutes, rinse the slice with tap water to terminate the color development, do not over-stain.
9. Wash the section with deionized water terminate the chromogenic reaction, then operate the procedures of counterstaining, dehydrating, transparentizing and sealing

Storage

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

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