2-step Plus Poly-HRP Anti Goat IgG Detection System（with DAB solution）

Cat. No: E-IR-R214 **Size: 3 mL/ 6 mL/ 18 mL**

DIA•AN® by Elabscience

Product Content

Cat	product	3 mL	6 mL	18 mL	Storage
E-IR-R214A	5% BSA	3 mL	6 mL	18 mL	2~8 ℃
E-IR-R214B	Polyperoxidase-anti-Goat IgG (Ready-to-Use)	3 mL	6 mL	18 mL	2~8 ℃
E-IR-R214C	3% H_2O_2	3 mL	6 mL	18 mL	2~8 ℃
E-IR-R214D	DAB Concentrate (20×)	150 μL	300 μL	900 μL	2~8 ℃
E-IR-R214E	DAB Substrate	3 mL	6 mL	18 mL	2~8 ℃
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/polyclonal antibody derived from goat.

Diluent for DAB concentrated solution has 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-R214C (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-R214A (5% BSA), incubate at room temperature for 30 min. Shake off any excess liquid.
5. Add primary antibody (Goat-IgG) with proper dilution ratio, incubate at 20~37 ℃ for 1~2h or at 4 ℃ overnight (then rewarm at 37 ℃ for 30 min). Wash with PBS or TBS, 2 min×3 times. Dry the section with absorbent paper.
6. Add E-IR-R214B (Polyperoxidase-anti-Goat IgG), incubate at room temperature or 37 ℃ for 20 min. Wash with PBS or TBS, 2 min×3 times.
7. Add 1 drop (approximately 50 μL) of E-IR-R214D (DAB Concentrate) into each 1 mL of E-IR-R214E (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. Take control of the DAB coloration period, the color of tan or brownish yellow is the positive signal. Avoid of excessive reaction.
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 ℃, shading light. Avoid of freezing. Valid for 12 months. The reagents are valid within 6 months after opening.