Ver.A261101

Immunohistochemistry kit

ORG-R217

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Immunohistochemistry kit 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 use 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.

Product components

Component	Volume
Normal Goat Blocking Buffer (Ready-to-use)	3mL
Polyperoxidase-anti-Mouse/Rabbit IgG (Ready-to-use)	3mL
3% H_2O_2	3mL
DAB Concentrate (25X)	120μL
DAB Substrate	3mL

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 3% H_2O_2 for 10 minutes to eliminate endogenous peroxidase activity. Wash with PBS or TBS, 2 minutes×3 times.
4. Add Normal Goat Blocking Buffer (Ready-to-Use), incubate at 37°C for 30 minutes. Shake off any excess liquid.
5. Add primary antibody (From Mouse or Rabbit) with proper dilution ratio, incubate at 20~37°C for 1-2 hours at 4°C overnight (then rewarm at 37°C for 30 minutes). Wash with PBS or TBS, 2 minutes×3 times. Dry the section with absorbent paper.
6. Add Polyperoxidase-anti-Mouse/Rabbit IgG, incubate at room temperature or 37°C for 20 minutes. Wash with PBS or TBS, 2 minutes×3 times.
7. Add 1 drop (approximately 40μL) of DAB Concentrate into each 1mL of 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 colour is obvious or the colour development time exceeds 10 minutes, rinse the slice with tap water to terminate the colour 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 freezing. Valid for 12 months. The reagents are valid within 6 months after opening.