

# Super Plus<sup>™</sup> Highly Sensitive and

## Rapid Immunohistochemical Kit (pH6.0)

Cat. No: E-IR-R221 Size: 3 mL/ 10 mL

#### **Product Content**

| Cat           | product  | 3 mL   | 10mL     | Storage |
|---------------|--|--------|----------|---------|
| SP Reagent 6A | Dewaxing/Antigen Retrieval Buffer(pH6.0) (20×) | 80 mL  | 120 mL×2 | 2~8°C   |
| SP Reagent B  | Peroxidase Blocking Buffer                     | 3 mL   | 10 mL    | 2~8°C   |
| SP Reagent C  | Polyperoxidase-anti-Rabbit/Mouse IgG           | 3 mL   | 10 mL    | 2~8°C   |
| SP Reagent D  | High Sensitive DAB Concentrate (20×)           | 150 μL | 500 μL   | 2~8°C   |
| SP Reagent E  | High Sensitive DAB Substrate                   | 3 mL   | 10 mL    | 2~8°C   |
| SP Reagent F  | Hematoxylin Staining Buffer                    | 3 mL   | 10 mL    | 2~8°C   |
| SP Reagent G  | Antibody Dilution Buffer                       | 3 mL   | 10 mL    | 2~8°C   |
| Manual        | One Copy                                       |        |          |         |

#### Introduction

Elabscience ® Super Plus<sup>TM</sup> Highly Sensitive and Rapid Immunohistochemical Kit is a high sensitive and rapid immunohistochemical broad spectrum detection reagent. There is no need to do the traditional three times of xylene dewaxing, three to five times of gradient ethanol hydration or antigen repair but only one Dewaxing/Antigen Retrieval Buffer heating without fume hood or traditional dewaxing/antigen repair tanks. The operation time is effectively shortened, the cumbersome operation steps are simplified so the variables in operation are reduced, and the stability is improved. The Dewaxing/Antigen Retrieval Buffer applies new environmental protection technology is not only reduces the harm to the body, but also cause no pollution to the environment.

This kit is easy to operate and fast because of the one-stop reagents used in the immunohistochemical experiment, and the high sensitive secondary antibody/ DAB detection reagents make this kit to be more sensitive than other produce.

This kit can be used to detect monoclonal/polyclonal antibody derived from Rabbit and Mouse.

#### Instructions

Dewaxing/Antigen Retrieval Buffer (pH6.0) ( $20\times$ ) [SP Reagent 6A] is a  $20\times$  concentrated solution. Dilute with DI water to  $1\times$  Dewaxing/Antigen Retrieval working solution before use.

For example: Take 10 mL Dewaxing/Antigen Retrieval Buffer (pH6.0) (20×), dilute with DI water to 200 mL.

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## Sample dyeing

- 1. Put the Dewaxing/Antigen Retrieval working solution in the repair box, then heat until it boils.
- 2. Put the slides into the boiling Dewaxing/Antigen Retrieval working solution to make the tissue immersed (In order to ensure the pH of the solution, the metal slide rack should not be used).
- 3. Repair the antigen with medium-low power for 15~30 min, and avoid the tissue from drying during the process. Take out the repair box to a RT environment, and it cool down naturally.
- 4. Take out the slides when the solution cooled down to RT, and wash the slides with DI water, make sure that there is no residue of Dewaxing/Antigen Retrieval working solution. (Don't flush the tissue directly during the washing process in order to avoid breaking up the tissue).
- 5. Dry the liquid around the tissue with absorbent paper, incubate with SP Reagent B (Peroxidase Blocking Buffer) at RT for 15 min to eliminate endogenous peroxidase activity. Wash with PBS or TBS, 2 min × 3 times.
- 6. Dry the PBS with absorbent paper, and draw a circle around the tissue with an oily pen. Add primary antibody with proper dilution ratio using SP Reagent G (Antibody Dilution Buffer), incubate at RT or 37°C for 30 min~1 h or at 4°C overnight (then rewarm at 37°C for 30 min). Wash with PBS or TBS, 2 min × 3 times.
- 7. Dry the PBS with absorbent paper, add a drop of SP Reagent C (Polyperoxidase-anti-Rabbit/Mouse IgG), incubate at RT or 37°C for 30 min. Wash with PBS or TBS, 2 min × 3 times.
- 8. Add 1 drop (approximately 50 μL) of SP Reagent D (High Sensitive DAB Concentrate) into each 1 mL of SP Reagent E (High Sensitive 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.
- 9. Dry the PBS with absorbent paper, Take control of the DAB coloration period, the color of tan or brownish yellow is the positive signal. Avoid of excessive reaction. Wash the section with DI water to terminate the chromogenic reaction.
- 10. Dry the liquid around the tissue with absorbent paper, incubate with SP Reagent F (Hematoxylin Staining Buffer) at RT for 5~10 min, and wash the slides with DI water.
- 11. Transfer slides into alkaline water for 1 min, then wash thoroughly with water for 5~10 min.
- 12. Dehydrate with gradient alcohol, transparent with transparent agent, drop Neutral Balsam beside the tissue, and then cover it with the cover glass and dry the slide.

#### Storage

Store at RT Valid for 12 months. The reagents are valid within 3 months after opening

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### **Cautions**

- 1. It is necessary to keep the slide wet during the operation. If the slide is dry, it will lead to non-specific staining results.
- 2. In the process of continuous heating, it is only necessary to keep boiling with medium-low power, and do not splash the Dewaxing/Antigen Retrieval working solution out of the repair box with high power.
- 3. The DAB should be well protected in the process of configuration and use to avoid the contact between the reagent and skin and eyes.

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