

## HRP Conjugated Anti-Human IgG SABC Kit

**Catalog No.** SA2004

**Size** 1 kit

### Product Type

Concentrated (Need dilution)

### Storage

4°C for one year. Avoid freezing.

### Tested Applications

Immunohistochemical analysis of paraffin-embedded sections, IHC(P);

Immunohistochemical analysis of frozen-embedded sections, IHC(F);

Immunocytochemistry, ICC.

### Recommended Dilution Factors

IHC(P): 1:50-200

IHC(F): 1:50-200

ICC: 1:50-200

Optimal dilutions should be determined by end users.

### Introduction

SABC (StreptAvidin-Biotin Complex) is specially designed for displaying the distribution of antigens in tissues and cells in immunochemistry and other immunodetection analyses. Streptavidin is a 47,000 dalton protein purified from the bacterium *Streptomyces avidinii*. Streptavidin has extraordinarily strong affinity to biotin molecules. The dissociation constant ( $K_d$ ) of the biotin-streptavidin complex is on the order of  $\sim 10^{-15}$  mol/L, a million times higher than the typical affinity between antigens and their antibodies. Streptavidin has very low non-specific binding to tissues and cells, due to its nearly neutral isoelectric point (IP=6.0~6.5). Therefore, immunohistochemical analyses based on streptavidin-biotin complex has extremely low background. Furthermore, this kit has high sensitivity because each complex it generates has a large number of peroxidase and streptavidin molecules. In brief, SABC offers high specificity, low background and ease-of-use.

### Kit Components

1. Normal rabbit serum blocking reagent: 10x, 10ml, for the block of tissue sections.
2. Biotinylated Secondary Antibody (Rabbit Anti-human IgG): 100~200x, 1ml (2mg/ml). Affinity purified antibody, labeled with "long-arm" biotin (Biotinamido hexanoic acid N-hydroxysuccinimide ester, CAS# 72040-63-2).
3. SABC-POD (Peroxidase conjugated streptavidin): 100~200x, 1ml (2mg/ml). Manufactured by Boster's proprietary method, the complex is very stable and offers superior amplification of the antigen signals.
4. Three drop bottles (For dilution use).

### Material Required But Not Provided

1. APES or POLY-L-LYSINE.
2. 0.02M PBS (pH 7.2~7.6): 8.5g sodium chloride, 2.8g anhydrous  $\text{Na}_2\text{HPO}_4$  and 0.4g anhydrous  $\text{NaH}_2\text{PO}_4$  in 1000ml of distilled water. (The weight should be adjusted accordingly if hydrous phosphates are used.)

- 0.01 M Citrate Buffer: 3g sodium citrate dihydrate ( $C_6H_5Na_3O_7 \cdot 2H_2O$ ) and 0.4g citric acid monohydrate ( $C_6H_8O_7 \cdot H_2O$ ) in 1000ml of distilled water.
- DAB Chromogenic Kit (Catalog number: AR1022 or AR1025).
- 0.1% trypsinase or the compound digest solution (Catalog number: AR0022).

### **Note**

Human IgG refers to the animal origin of the primary antibody, not the origin of the specimen. This kit must be used on primary antibodies from human.

### **Options of immunohistochemistry staining process**

The best process among the following may have to be identified by trial and error. The characteristics of the antigen/antibody used may be used as a guideline.

#### **A. Heat repair antigen process**

Applies to immunohistochemical analysis of paraffin-embedded sections, to expose the antibody binding site on the antigens.

#### **B. Enzyme digestion process**

Applies to immunohistochemical analysis of paraffin-embedded sections, to expose the antibody binding site on the antigens.

#### **C. Non-digestion/non-repair process**

Applies to stable antigens using immunohistochemical analysis of paraffin-embedded sections.

#### **D. Blood smear, cultured cells and frozen section staining process**

Applies to immunocytochemistry of blood smear and cultured cells, and immunohistochemical analysis of frozen-embedded sections.

### **Assay Procedure**

#### **A. Heat repair antigen process**

- Cover the entire surface of a clean microslide with APES (Catalog number: AR0001) or POLY-L-LYSINE (Catalog number: AR0003). Incubate for 1 minute then rinse the microslide with water. Mount a tissue section (~5 $\mu$ m thick) with the treated microslide and bake in an oven at 58-60 °C for 30-60 minutes to ensure strong adhesion of the tissue section.
- Dewax the tissue section in dimethylbenzene for 10 minutes and rinse with water.
- Incubate the tissue section for 5~10 minutes in the 3%  $H_2O_2$  solution to quench the endogenous peroxidase activity. Wash the tissue section with distilled water 3 times for 2 minutes each.
- To heat repair the antigen, soak the tissue section in 0.01M citrate buffer (pH6.0), and heat to the boiling point with an electric heater or a microwave oven, then stop heating. Repeat this heating process 1~2 times with a 5~10-minute interval. Wash the tissue section with 0.02 M PBS (pH 7.2~7.6) once or twice when it cools at room temperature.
- Add normal rabbit serum blocking reagent to the tissue section and incubate at room temperature for 20 minutes. Discard the blocking reagent solution, but do not wash the tissue section.
- Add properly diluted primary antibody (human IgG) to the tissue section and incubate at 37 °C for about 1 hour or 20 °C for about 2 hours or at 4 °C overnight. Wash the tissue section with 0.02M PBS (pH 7.2~7.6) 3 times for 2 minutes each. (The primary antibody concentration, incubation time and temperature directly affect the staining efficiency and background intensity. If the positive staining is too weak, the concentration of the primary antibody and the incubation time can be increased; if the background is too high, the primary antibody concentration and the incubation time can be decreased. )

7. Add biotinylated rabbit anti-human IgG to the tissue section and incubate at 20~37°C for 20 minutes. Wash the tissue section with 0.02M PBS (pH 7.2~7.6) 3 times for 2 minutes each.
8. Add SABC-Peroxidase (Streptavidin-Peroxidase) to the tissue section and incubate at 20~37°C for 20 minutes. Wash the tissue section 4 times with 0.02M PBS (pH 7.2~7.6) for 5 minutes each.
9. Use a DAB chromogenic kit (Catalog number: AR1022 or AR1025) to stain the tissue section. Add Reagent A, B and C, one drop each, into 1 ml of distilled water and mix thoroughly. Add this solution to the tissue section and incubate at room temperature. Control the time of incubation under a microscope. Usually 5~30 minutes is sufficient. Wash the tissue section with distilled water.
10. Slightly counterstain the tissue section with haematoxylin(if use AR1022) or nuclear fast red(if use AR1025), and wash with distilled water to clean the haematoxylin. Then dry the tissue section by baking, and put on a drop of resin seal the tissue section with a cover slide. The tissue section is ready for observation under a microscope.

#### **B. Enzyme digestion process**

The enzyme digestion process is similar to the heat repair antigen process. Simply replace the 4<sup>th</sup> step in the heat repair antigen process with the following.

- Incubate the tissue section in 0.1% trypsinase or compound digestive solution (Catalog number: AR0022) for 5~10 minutes. Wash with distilled water 3 times.

#### **C. Non-digestion/non-repair process**

The process is for antigens which do not need heat repair or digestion. Simply omit the 4<sup>th</sup> step in the heat repair antigen process.

#### **D. Blood smear, cultured cells or frozen sections staining process**

1. Treat a microslide with POLY-L-LYSINE as described in Process A.
  - Blood samples. Add anticoagulant to the samples and smear the blood samples onto the treated microslide.
  - Cultured cells. Cultured cells can be smeared onto or directed cultivated on the treated microslide.
  - Sections of frozen tissue. Sections of frozen tissue may be placed onto the treated microslide and air-dry at room temperature for 30 minutes until no liquid water is visible.
2. Fix the sample with 4% paraformaldehyde or 10% formalin for 60~90 minutes.
3. Dilute 30% H<sub>2</sub>O<sub>2</sub> at 1:50 with pure methanol. Incubate the fixed sample for 30 minutes in the diluted H<sub>2</sub>O<sub>2</sub> to quench the endogenous peroxidase activity. Wash the sample with distilled water once or twice. If the direct staining result of frozen tissue sections is not satisfactory, the tissue sections may be repaired by following the 4<sup>th</sup> step in the heat repair antigen process.
4. Follow steps 5-10 in the heat repair antigen process.

#### **Note**

1. If the staining background is too high, wash the section with 0.01-0.02% TWEEN 20 PBS (pH7.2-7.6) 4 times and with pure PBS twice after SABC reaction and before DAB staining, then use DAB chromogenic kit to stain the section.
2. 0.01M citrate buffer (pH 6.0), PBS, or TBS buffer may be used to repair the section.