

## Cy3 Conjugated Rat IgG SABC Kit

**Catalog No.** SA1079

**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. This kit has high sensitivity because each complex it generates has a large number of Cy3 and streptavidin molecules. Compared to the traditional immunodetection using free Cy3 dyes, the SABC-Cy3 system greatly enhances the sensitivity and suppresses the background. Cy3 is activated at 554nm, fluorescing at 568~574nm with bright red.

### Kit Components

1. Normal rabbit serum blocking reagent: 10x, 5 ml, for the block of tissue sections.
2. Biotinylated Secondary Antibody (Rabbit Anti-rat IgG): 100x, 0.5 ml (2mg/ml). Affinity purified antibody, labeled with "long-arm" biotin (Biotinamido hexanoic acid N-hydroxysuccinimide ester, CAS# 72040-63-2).
3. SABC-Cy3 (Cy3 conjugated streptavidin): 100x, 0.5ml (1mg/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 Na<sub>2</sub>HPO<sub>4</sub> and 0.4g anhydrous NaH<sub>2</sub>PO<sub>4</sub> in 1000ml of distilled water. (The weight should be adjusted accordingly if hydrous phosphates are used.)
3. 0.01 M Citrate Buffer: 3g sodium citrate dihydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>·2H<sub>2</sub>O) and 0.4g citric acid monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>·H<sub>2</sub>O) in 1000ml of distilled water.
4. 0.1% trypsinase or the compound digest solution (Catalog number: AR0022).

**FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC AND CLINICAL USE.**

Product Information Sheet

### **Note**

Rat 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 rat.

### **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. Paraffin section staining process**

Applies to immunohistochemical analysis of paraffin-embedded sections.

#### **B. 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. Paraffin section staining process**

1. 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.
2. Dewax the tissue section in dimethylbenzene for 10 minutes and rinse with water.
3. To heat repair the antigen if there is a need, soak the tissue section in 0.01M citrate buffer (pH 6.0), and heat to the boiling point with an electric heater or a microwave oven, then stop heating. Repeat this heating process 2~3 times with a 5~10-minute interval. Wash the tissue section with distilled water 3 times for 2 minutes each.
4. Dilute the normal rabbit serum blocking reagent at 1:10 with 0.02M PBS (pH 7.2~7.6). Add the diluted 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.
5. Add properly diluted primary antibody (rat 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.
6. Add biotinylated rabbit anti-rat IgG (diluted at 1:100 with 0.02M PBS (pH 7.2~7.6)) to the tissue section and incubate at 20~37°C for 30 minutes. Wash the section with 0.02 M PBS 3 times for 2 minutes each.
7. Dilute SABC-CY3 (Streptavidin-CY3) at 1:100 with 0.02M PBS (pH 7.2~7.6). Add the diluted SABC-CY3 solution to the tissue section and incubate at 20~37°C for 30 minutes. Wash the tissue section 4 times with 0.02M PBS (pH 7.2~7.6) for 5 minutes each.
8. Put several drops of the water soluble sealing reagent onto the tissue section and seal with a cover slide. The tissue section is ready for observation under a fluorescence microscope.

#### **B. Blood smear, cultured cells and frozen section 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 3 times for 2 minutes each. 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 4-8 in the heat repair antigen process.