BOSTER
antibody and ELISA experts

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FITC + POD Conjugated Anti-Human IgG SABC Kit

Catalog No. SA1084

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 FITC or peroxidase, and streptavidin molecules. Compared to the traditional immunodetection using free FITC dyes, the SABC-FITC system greatly enhances the sensitivity and suppresses the background. FITC excitation is at 490~495nm, emission at 520~530nm.

Kit Components

1. Normal rabbit serum blocking reagent: 10×, 5 ml, for blocking tissue sections.
2. Biotinylated Secondary Antibody (Rabbit Anti-human IgG): 100×, 0.5ml (2mg/ml). Affinity purified antibody, labeled with “long-arm” biotin (Biotinamidohexanoic acid N-hydroxysuccinimide ester, CAS# 72040-63-2).
3. SABC-FITC+Peroxidase (FITC and peroxidase conjugated streptavidin): 100×, 0.5ml (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 Na_2HPO_4 and 0.4g anhydrous NaH_2PO_4 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 ($C_6H_5Na_3O_7·2H_2O$) and 0.4g citric acid monohydrate ($C_6H_8O_7·H_2O$) in 1000ml of distilled water.
4. DAB Chromogenic Kit (Catalog number: AR1022 or AR1025).
5. 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

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 (~5μm 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. Incubate the tissue section for 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.
4. To heat repair the antigen, 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. Allow the sample to cool down.
5. Dilute the normal rabbit serum blocking reagent with 0.02M PBS (pH 7.2~7.6) at 1:10. 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.
6. 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. Dilute the concentrated secondary antibody (biotinylated rabbit anti-human IgG) with 0.02M PBS (pH 7.2~7.6) at 1:100. Incubate the tissue section with the diluted secondary antibody at 20~37˚C for 20 minutes. Wash the tissue section with 0.02M PBS (pH 7.2~7.6) 4 times for 5 minutes each.
8. Dilute the concentrated SABC-FITC+Peroxidase with 0.02M PBS (pH 7.2~7.6) at 1:100. Incubate the tissue section in the diluted SABC-FITC +Peroxidase at 20~37˚C for 20 minutes. Then wash 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. Positively staining appears yellow green under fluorescence microscope and brown under light microscope.

B. Enzyme digestion process

The enzyme digestion process is similar to the heat repair antigen process. Simply replace the 4th 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 4th step in the heat repair antigen process.

D. 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 to fix tissue section.
2. Fix the sample with 4% paraformaldehyde or 10% formalin for 60~90 minutes at room temperature.
3. Dilute 30% H_2O_2 at 1:50 with pure methanol. Incubate the fixed sample for 30 minutes in the diluted H_2O_2 to quench the endogenous peroxidase activity. Wash with distilled water 1~2 times.
4. Follow steps 5-10 in the heat repair antigen process.

Notice

1. Under fluorescence microscope: yellow green particles are the positive staining.
2. Under light microscope: brown particles are the positive staining.