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Alkaline Phosphatase Conjugated Rat IgG SABC Kit

Catalog No. SA1055

Size 1 kit

Product Type

Ready to use (No dilution needed)

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.

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 ~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 alkaline phosphatase and streptavidin molecules. In brief, SABC offers high specificity, low background and ease-of-use.

Kit Components

1. Normal rabbit serum blocking reagent: 12 ml, for blocking tissue sections.
2. Biotinylated Secondary Antibody (Rabbit Anti-rat IgG): 12ml (10μg/ml). Affinity purified antibody, labeled with “long-arm” biotin (Biotinamidohexanoic acid N-hydroxysuccinimide ester, CAS# 72040-63-2).
3. SABC-AP (Alkaline Phosphatase conjugated streptavidin): 12ml (20μg/ml). Manufactured by Boster’s proprietary method, the complex is very stable and offers superior amplification of the antigen signals.
4. BCIP/NBT Chromogenic agent (20×): 1ml.
5. Water soluble sealing reagent: 12 ml.
6. Neutral nuclear fast red: 12 ml.

Material Required But Not Provided

1. APES or POLY-L-LYSINE.
2. 0.01M TBS (pH 7.2~7.4): 9g sodium chloride, 1.2g Tris, and 450μl acetic acid in 1000ml of distilled water.
3. 0.01M TBS (pH 9.0~9.5): 9g sodium chloride and 1.2g Tris in 1000ml of distilled water.
4. 0.01M 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.
5. 0.1% trypsinase or the compound digest solution (Catalog number: AR0022).

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. 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. 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 0.01 M TBS (pH7.2~7.4) once or twice when it cools to room temperature.
4. Add 5% BSA blocking reagent solution 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 0.01M TBS (pH7.2-7.4) 3 times for 2 minutes each.
6. Add biotinylated rabbit anti-rat IgG to the tissue section and incubate at 20~37˚C for 20 minutes. Wash the tissue section with 0.01M TBS (pH7.2~7.4) 3 times for 2 minutes each.
7. Add SABC-AP (Streptavidin-AP) to the tissue section and incubate at 20~37˚C for 20 minutes. Wash the tissue section 4 times with 0.01M TBS (pH7.2~7.4) for 5 minutes each. Wash with water once.
8. Use a BCIP/NBT chromogenic kit (Catalog number: AR1023) to stain the tissue section. Dilute the BCIP/NBT concentrated solution at 1:20 with 0.01 M TBS (pH 9.0~9.5). Add this diluted solution to the tissue section and incubate at 20~37˚C. Control the time of incubation under a microscope. Usually 10~30 minutes is sufficient. Wash the tissue section with distilled water.
9. Slightly counterstain the tissue section with nuclear fast red. Wash the tissue section with water. Then dry the tissue section by baking, and put on several drops of water soluble sealing reagent (Heat this reagent to 60℃) onto the tissue section and seal 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 3th 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 3th 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. Incubate the fixed sample for 20 minutes in 3% glacial acetic acid at room temperature to quench the endogenous peroxidase activity. Wash the sample with distilled water once or twice. If the direct staining result of frozen section is not satisfactory, the tissue sections may be repaired by following the 3th step in the heat repair antigen process.
4. Follow steps 4-9 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 TBS (pH7.2-7.4) 4 times with pure PBS twice after SABC reaction and before BCIP/NBT staining, then use BCIP/NBT chromogenic kit to stain the section.
2. 0.01M citrate buffer (p6.0), PBS, or TBS buffer may be used to repair the section.