Rabbit IgG SABC Kit

Cat#: orb90444 (ELISA Manual)

Catalog No. orb90444
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 (Strept Avidin-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 (Kd) 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 peroxidase and streptavidin molecules. In brief, SABC offers high specificity, low background and ease-of-use.

Kit Components

1. 5% BSA Blocking Reagent: 12 ml, for blocking tissue sections.

2. Biotinylated Secondary Antibody (Goat Anti-rabbit IgG): 12ml (10μg/ml). Affinity purified antibody, labeled with “long-arm” biotin (Biotin amid ohexanoic acid N-hydroxy succinimide ester, CAS# 72040-63-2).

3. SABC-POD (Peroxidase conjugated streptavidin): 12ml (20μg/ml). Manufactured by Biorbyt’s proprietary method, the complex is very stable and offers superior amplification of the antigen signals.

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 ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 0.4g citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in 1000ml of distilled water.

4. DAB Chromogenic Kit.

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5. 0.1% trypsinase or the compound digest solution.

Note Rabbit 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 rabbit.

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 or POLY-L-LYSINE. 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 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.

4. 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 to room temperature.

5. 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.

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6. Add properly diluted primary antibody (rabbit 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 goat anti-rabbit 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 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 hematoxylin or nuclear fast red and wash with distilled water to clean the hematoxylin. 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 4th step in the heat repair antigen process with the following.

Incubate the tissue section in 0.1% trypsinase or compound digestive solution 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 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_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 the sample with distilled water once or twice. If the direct

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staining result of frozen tissue sections is not satisfactory, the tissue sections may be repaired by following the 4th 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.