Goat Specific Cy3/SABC Detection Kit – KOA0119

Goat Specific Cy3/SABC kit is a streptavidin-biotin immuno-enzymatic antigen detection system. This system involves the sequential incubation of the sample with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody that reacts with the primary antibody, enzyme-labeled streptavidin, and chromogen substrate.

I. KIT COMPONENTS

Goat Specific Cy3/DAB (ABC) Detection Kit			
Component	**Catalog Number**	**Description**	**Size**
Blocking Reagent	KOB0102	Normal Rabbit Serum (10X)	5ml
Biotinylated Secondary Antibody	KOK0606	Anti-Goat IgG (Rabbit) Biotin Conjugated Secondary (100X)	0.5mL
SABC-Cy3	KOS0401	Cy3 Conjugated Streptavidin (100X)	0.5mL
Drop Bottles	KOJ0101	For Dilution	3

II. STORAGE

Store at 4°C for one year. Avoid freezing.

III. INTRODUCTION

StreptAvidin-Biotin Complex (SABC) is specially designed to display the distribution of antigens in tissues and cells in immunochemistry and other immunodetection analyses. This kit has high sensitivity as each complex it generates has a large number of Cy3 and streptavidin molecules. Compared to traditional immunodetection methods that use free Cy3 dyes, the SABC-Cy3 system greatly enhances sensitivity and suppresses background. Cy3 is activated at a wavelength of 554nm and fluoresces bright red at ~568-574nm with bright red.

IV. REQUIRED EQUIPMENT AND REAGENTS

- APES or POLY-L-LYSINE
- 20mM PBS (pH 7.2-7.6)
  - 9.0g sodium chloride, 1.2g Tris, and 450µl acetic acid in 1000ml of distilled water. (The weight should be adjusted accordingly if hydrous phosphates are used.)
- 0.01 M Citrate Buffer
  - 3.0g sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 0.4g citric acid monohydrate ($C_6H_5Na_3O_7 \cdot H_2O$) in 1000ml of distilled water.
- 0.1% trypsinase or the compound digest solution

V. IHC PROTOCOL

Note: Goat 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 goat.

A. Options For Immunohistochemistry Staining Process

The best process amongst the following may have to be identified by the end user. The characteristics of the antigen/antibody used may be followed as a guideline.

- ### Heat-induced antigen retrieval process
  Heat-induced antigen retrieval applies to immunohistochemical analysis of paraffin-embedded sections to expose the antibody binding site on the antigens.

- ### Enzyme digestion process
  Enzyme digestion process applies to immunohistochemical analysis of paraffin-embedded sections to expose the antibody binding site on the antigens.

- ### Non-digestion/non-retrieval process
  Non-digestion/non-repair process applies to stable antigens using immunohistochemical analysis of paraffin-embedded sections.

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

B. Assay Procedure

- ### Heat-induced antigen retrieval process

1. Cover the entire surface of a clean microslide with APES or POLY-L-LYSINE. Incubate for 1 minute and then rinse the microslide with water. Mount a tissue section (~5μm thick) onto 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. For heat-induced antigen retrieval, add a sufficient volume of 0.01M citrate buffer (pH 6.0) in a microwavable container and heat the buffer to 90˚-100˚C in a microwave. Place the slides in the container with the heated citrate buffer (the buffer should cover the slides by at least a few centimeters) and place the container inside the microwave. Set the microwave to full power and boil for 5-10 minutes. Remove the container from the microwave and allow the slides to cool at room temperature for 15-20 minutes.
4. 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.
5. Add properly diluted primary antibody (goat 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.
6. Wash with 20mM PBS (pH 7.2-7.4) 3 times for 5 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-goat IgG to the tissue section and incubate at 20-37˚C for 20 minutes. Wash the tissue section with 20mM PBS (pH=7.2-7.6) 3 times for 2 minutes each.
8. Add SABC-CY3 (Streptavidin-CY3) 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 SABC-CY3 to stain the tissue section to stain the tissue section. Dilute the SABC-CY3 concentrated solution at 1:100 with 20mM PBS (pH=7.2-7.6). 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.
10. Wash the tissue section with distilled water.
11. Slightly counterstain the tissue section with hematoxylin or nuclear fast red and wash with distilled water to clean the hematoxylin. Dry the tissue section by baking, and put on a drop of resin seal. The tissue section is ready for observation under a microscope.

- ## Enzyme digestion process

The enzyme digestion process is similar to the heat-induced antigen retrieval process. Simply replace step 4 from the heat-induced retrieval process with the following:

- Incubate the tissue section in 0.1% trypsinase or compound digestive solution for 5-10 minutes. Wash three times with distilled water and continue with the immunostaining protocol as above.

- ## Non-digestion/non-retrieval process

This process is for antigens that do not need heat retrieval or digestion. Simply omit step 4 and continue with the immunostaining protocol.

- ## Blood smear, cultured cells or frozen sections staining process

1. Treat a microslide with POLY-L-LYSINE as described above.
    - Blood samples: Add anticoagulant to the samples and smear the blood samples onto the treated slide.
    - Cultured cells: Cultured cells can be smeared onto or directly cultivated on the treated slide.
    - Frozen tissue sections: Sections of frozen tissue may be placed onto the treated slide and air-dry at room temperature for 30 minutes until no liquid 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 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 step 4 from the heat repair antigen process.
4. Follow steps 4-8 from the heat repair antigen process.

    Note:

    - If the staining background is too high, wash the section with 0.01-0.02% TWEEN 20-PBS (pH=7.2-7.4) 4 times and then with PBS twice after SABC reaction and before BCIP/NBT chromogenic kit to stain this section.

- 0.01M citrate buffer (pH=6.0), PBS, or TBS buffer may be used to repair the section.

VI. TROUBLESHOOTING

Weak or No Signal

Possible Cause	Solution
Slides lose signal over time during storage	a) Prepare slides with freshly-sectioned tissues b) Store slides at 4°C c) Do not bake slides before storage
The antibody used is not suitable for IHC procedures which detect proteins in its native conformation	a) Check the antibody datasheet to make certain that it has been validated for IHC applications b) Check if the antibody is applicable for the right IHC samples (paraffin sections vs. frozen samples) c) Perform Western blot in both its native and denatured forms to ensure that the antibody detects the native form
Fixation procedures (using formalin/paraformaldehyde fixatives) have masked the epitope that the antibody recognizes	a) Use different antigen retrieval methods to unmask the epitope (HIER or PIER) b) Fix the sections in a shorter time
The primary and/or secondary antibody has lost its activity due to improper storage, dilution or excessive freezing and thawing	a) Run positive controls to ensure that the primary and/or secondary antibody is working properly b) Store the antibodies per manufacturer instructions c) Avoid contamination of antibodies and exposure to light
Insufficient deparaffinization	a) Increase the deparaffinization time b) Use fresh dimethylbenzene
The protein is located in the nucleus and the antibody cannot penetrate the nucleus	a) Add a permeabilizing agent (e.g. Triton X) to the blocking buffer and antibody dilution buffer
The PBS buffer is contaminated with bacteria that damage the phosphate groups on the protein of interest	a) Add 0.01% azide in the PBS antibody storage buffer Use fresh sterile PBS
The primary and the secondary antibodies are not compatible	a) Use a secondary antibody that was raised against the species in which the primary was raised (e.g. if the primary antibody was raised in mouse, an anti-mouse secondary antibody should be used) b) Check that the isotypes of the primary and secondary antibodies are compatible
The protein is not present in the tissue of interest or is not sufficiently expressed	a) Run positive controls to ensure that target protein is present in the tissue b) Include an amplification step in your protocol c) Use higher antibody concentration
Insufficient antibody to detect protein of interest	a) Use a higher antibody concentration b) Incubate for a longer time (e.g. overnight at 4°C)

Tissue has dried out	a) Cover the tissue sections in liquid at all time during the experiment

High Background

Possible Cause	Solution
The blocking buffer is incorrect	a) Make sure to use the blocking buffer recommended by the manufacturer
Blocking is insufficient (Do not over-block the tissue because antigenic sites may be masked)	a) Increase blocking time b) Change blocking reagent: i. For tissue sections, use 10% normal serum (1 hour) ii. For cell cultures, use 1-Normal Rabbit Serum (0.5 hours)
The primary antibody concentration is too high	a) Titrate the antibody to determine the optimal concentration b) Incubate at 4°C
Non-specific binding by secondary antibody	a) Run a secondary control without primary antibody: If you see staining with your secondary only: i. Change secondary antibody or ii. Use secondary antibody that has been pre-adsorbed b) Block sample with serum from the same species as the host in which the secondary antibody was raised
Endogenous peroxide or phosphatase is active	a) Quench the endogenous peroxidase or phosphatase activity by enzyme inhibitors: i. Peroxidase: use H_2O_2 and methanol (v/v: 0.3%: 99.7%) ii. Phosphatase: 2mM Levamisol
Tissue section is too thick for reagent penetration	Prepare thinner section
Too much substrate was applied (enzymatic detection)	a) Dilute substrate b) Reduce substrate incubation time c) Choose substrate of higher S/N ratio e.g. Metal-enhanced DAB
Incubation temperature is too high	a) Incubate samples at 4°C
Primary antibody was raised in the same species as source of tissue (therefore, secondary antibody recognizes and binds non-specifically to the tissue)	a) Use primary antibody raised against a species which is different from the source of tissue b) Use biotinylated primary antibody and conjugated streptavidin for the detection system
Secondary antibody binds endogenous IgG	a) Include control slide stained without the primary antibody to confirm whether the secondary antibody is the source of the background
Fixation reagents are still present (Due to insufficient tissue washing)	a) Wash the tissues extensively with PBS buffer
Reaction between chromogens and PBS buffer in tissue or cell samples	a) Before incubating with the substrate, use Tris buffer to wash the samples

Membrane damage by permeabilization	a) Use a less stringent detergent such as Tween 20 instead of Triton X b) Remove permeabilizing agent from your buffers
Insufficient deparaffinization	a) Increase the deparaffinization time b) Use fresh dimethylbenzene
High levels of endogenous biotin in biotin-based detection systems for samples (e.g. liver and kidney tissues)	a) Perform biotin block after normal blocking procedure and before primary antibody incubation b) Use polymer-based detection

VII. RELATED PRODUCTS

Component	Catalog #	Size
10x TBS pH 7.5	MB-012	1000ml
10x TTBS pH 7.5	MB-013	1000ml
10X PBS pH 7.2	MB-008	1000ml
10x PBST pH 7.2	MB-075-1000	1000ml