Mouse Specific Cy3/SABC Detection Kit – KOA0116

Mouse Specific Cy3/SABC Detection 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

Mouse Specific Cy3/DAB (ABC) Detection Kit			
Component	Catalog Number	Description	Size
Blocking Reagent	KOB0101	Normal Goat Serum Blocking Reagent	5mL
Biotinylated Secondary Antibody	KOK0601	Anti-Mouse IgG (Goat) Biotinylated Conjugated Antibody	0.5mL
SABC-Cy3	KOS0401	Cy3 Conjugated Streptavidin (100-200X)	0.5mL
Dropper Bottles	KOJ0101	Dropper Bottles (empty for dilution)	3

II. STORAGE

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

III. INTRODUCTION

StreptAvidin-Biotin Complex (SABC) is specially designed for displaying 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 using free Cy3 dyes, the SABC-Cy3 system greatly enhances sensitivity and suppresses background. Cy3 is activated at 554nm, fluorescing bright red between 568 - 574nm.

IV. REQUIRED EQUIPMENT AND REAGENTS

- APES or POLY-L-LYSINE
- 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.)
- 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: Mouse 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 mouse.

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

Paraffin section staining 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. 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 goat serum blocking reagent at 1:10 with 0.02M PBS (pH7.2-7.6). 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 (mouse 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 goat anti-mouse 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 (Streptavidin-CY3) at 1:100 with 0.02M PBS (pH 7.2 - 7.6). Add the diluted Streptavidin-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 now ready for observation under a fluorescence microscope.

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-dried 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 sections is not satisfactory, the tissue sections may be repaired by following step 4 of the antigen retrieval process.
4. Follow steps 4-8 from the heat-induced antigen retrieval process.

VI. TROUBLESHOOTING

Weak or No Signal

Possible Cause	Solution
Slides lose signal over time during storage	Prepare slides with freshly-sectioned tissues Store slides at 4°C Do not bake slides before storage
The antibody used is not suitable for IHC procedures which detect proteins in its native conformation	Check the antibody datasheet to make certain that it has been validated for IHC applications Check if the antibody is applicable for the right IHC samples (paraffin sections vs. frozen samples) 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	Use different antigen retrieval methods to unmask the epitope (HIER or PIER) 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	Run positive controls to ensure that the primary and/or secondary antibody is working properly Store the antibodies per manufacturer instructions Avoid contamination of antibodies and exposure to light
Insufficient deparaffinization	Increase the deparaffinization time Use fresh dimethylbenzene

The protein is located in the nucleus and the antibody cannot penetrate the nucleus	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 phosphate groups on the protein of interest	Add 0.01% azide in the PBS antibody storage buffer Use fresh sterile PBS
The primary and the secondary antibodies are not compatible	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) 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	Run positive controls to ensure that target protein is present in the tissue Include an amplification step in your protocol Use higher antibody concentration
Insufficient antibody to detect protein of interest	Use a higher antibody concentration Incubate for a longer time (e.g. overnight at 4°C)
Tissue has dried out	Cover the tissue sections in liquid at all time during the experiment

High Background

Possible Cause	Solution
The blocking buffer is incorrect	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)	Increase blocking time Change blocking reagent: (a) For tissue sections, use 10% normal serum (1 hour) (b) For cell cultures, use 1-5% BSA (0.5 hours)
The primary antibody concentration is too high	Titrate the antibody to determine the optimal concentration Incubate at 4°C
Non-specific binding by secondary antibody	Run a secondary control without primary antibody: If you see staining with your secondary only: (a) Change secondary antibody or (b) Use secondary antibody that has been pre-adsorbed Block sample with serum from the same species as the host in which the secondary antibody was raised
Endogenous peroxide or phosphatase is active	Quench the endogenous peroxidase or phosphatase activity by enzyme inhibitors: (a) Peroxidase: use H_2O_2 and methanol (v/v: 0.3%: 99.7%) (b) Phosphatase: 2mM Levamisol
Tissue section is too thick for reagent penetration	Prepare thinner section
Too much substrate was applied (enzymatic detection)	Dilute substrate Reduce substrate incubation time Choose substrate of higher S/N ratio e.g. Metal-enhanced DAB
Incubation temperature is too high	Incubate samples at 4°C
Primary antibody was raised in the same species as source of tissue (therefore, secondary antibody	Use primary antibody raised against a species which is different from the source of tissue

recognizes and binds non-specifically to the tissue)	Use biotinylated primary antibody and conjugated streptavidin for the detection system
Secondary antibody binds endogenous IgG	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)	Wash the tissues extensively with PBS buffer
Reaction between chromogens and PBS buffer in tissue or cell samples	Before incubating with the substrate, use Tris buffer to wash the samples
Membrane damage by permeabilization	Use a less stringent detergent such as Tween 20 (instead of Triton X) Remove permeabilizing agent from your buffers
Insufficient deparaffinization	Increase the deparaffinization time Use fresh dimethylbenzene
High levels of endogenous biotin in biotin-based detection systems for samples (e.g. liver and kidney tissues)	Perform biotin block after normal blocking procedure (before primary antibody incubation) 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