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FITC Conjugated Anti-Mouse IgM SABC Kit

Catalog No. SA1063

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 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 goat serum blocking reagent: 10×, 10 ml, for the block of tissue sections.
2. Biotinylated Secondary Antibody (Goat Anti-mouse IgM): 100~200×, 1 ml (2mg/ml). Affinity purified antibody, labeled with “long-arm” biotin (Biotinamidohexanoic acid N-hydroxysuccinimide ester, CAS# 72040-63-2).
3. SABC-FITC (FITC conjugated streptavidin): 100~200×, 1ml (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 ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 0.4g citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) in 1000ml of distilled water.
4. 0.1% trypsinase or the compound digest solution (Catalog number: AR0022).

Note

Mouse IgM 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.

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. Paraffin section staining process

Applies to immunohistochemical analysis of paraffin-embedded sections.

B. 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. Paraffin section staining 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 (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 IgM) 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 IgM (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 SABC-FITC (Streptavidin-FITC) at 1:100 with 0.02M PBS (pH 7.2~7.6). Add the diluted SABC-FITC 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 7.2~7.4) 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 ready for observation under a fluorescence microscope.

B. 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.
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 the 4th step in the heat repair antigen process.
4. Follow steps 4-8 in the heat repair antigen process.