Ready-to-Use SABC Kit (Mouse)

M777864

Storage Store at 2-8℃.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

This immunohistochemistry kit adopts the SABC amplification system with DAB chromogenic detection. SABC is designed for immunohistochemistry and other immunoassays to visualize antigen distribution in tissues and cells.

Streptavidin is a protein extracted from Streptomyces with a molecular weight of 47 000. Similar to avidin, it possesses extremely high affinity for biotin, approximately one-million-fold higher than typical antigen-antibody affinity. Avidin is a basic protein (IP = 10) and can be modified into a neutral form. Streptavidin has a near-neutral isoelectric point (IP = 6.0-6.5) and exhibits very low non-specific adsorption to tissues and cells, which enables low-background staining in streptavidin-based immunohistochemical methods.

SABC stands for StreptAvidin-Biotin Complex. Studies show each SABC complex contains roughly 100 peroxidase molecules and around 50 streptavidin molecules. The abundant enzyme moiety delivers high assay sensitivity. Therefore, the SABC method features high sensitivity, low background and simple operation.

Kit Contents

M777864	Components	1 Kit	Storage
M777864A	Normal Rabbit Serum Blocking Solution	6mL	2-8℃.
M777864B	Biotin-Goat Anti Mouse IgG	6mL	2-8℃.
M777864C	SABC Complex	6mL	2-8℃.

Required Reagents & Consumables (Not Supplied)

1. Adhesion-coated glass slides.
2. PBS (pH 7.2-7.6).
3. EDTA antigen retrieval buffer or citrate buffer.
4. Endogenous peroxidase blocking reagent.
5. DAB Chromogenic Reagent Kit (D1505853).

Operating Procedures

A. Heat-Induced Antigen Retrieval for Paraffin Sections

1. Prepare sections and perform routine de-paraffinization to hydration.
2. Apply endogenous peroxidase blocking reagent; incubate at room temperature for 5-10 min to block endogenous peroxidase activity. Rinse with distilled water 3 times.
3. Heat-induced antigen retrieval: Immerse sections in EDTA antigen retrieval buffer or citrate buffer. Heat in microwave or electric heater until boiling, then power off. Keep interval of 5-10 min and repeat heating-cooling cycle 1-2 times. Cool down, then wash with PBS 1-2 times.
4. Add Normal Rabbit Serum Blocking Solution. Incubate at room temperature for 20 min. Remove excess liquid; do not wash.
5. Add appropriately diluted primary antibody. Incubate at 37 ℃ for ~1h, or at 20 ℃ for ~2h; alternatively incubate at 4 ℃ overnight. Wash with PBS for 2 min × 3 times.

Note: Primary-antibody dilution, incubation temperature and duration directly affect staining intensity and background. If positive signal is weak, increase primary-antibody concentration or prolong incubation time. If high background occurs, reduce primary-antibody concentration or shorten incubation time.

6. Add Biotin-Goat Anti Mouse IgG. Incubate at 20-37 ℃ for 20 min. Wash with PBS for 2 min × 3 times.
7. Add SABC complex reagent. Incubate at 20-37 ℃ for 20 min. Wash with PBS for 5 min × 4 times.
8. DAB chromogenic reaction: Prepare DAB working solution: Mix DAB-A and DAB-B thoroughly at a ratio of 1:1 to prepare the DAB working solution. It is recommended to use it within 4 hours. Apply onto sections. Develop at room temperature and monitor reaction under microscope (typically 1-15 min). Terminate reaction by rinsing with distilled water.
9. Counterstain lightly with hematoxylin. Dehydrate, clear, mount slides, and observe under microscope.

B. Enzymatic Digestion Protocol for Paraffin Sections

Replace Step 3 in Protocol A with the following step:

Add compound digestive solution and incubate for 5-10 min. Rinse with distilled water 3 times. Alternatively, 0.1 % trypsin solution can be used for digestion.

C. Protocol without Digestion / Antigen Retrieval for Paraffin Sections

For antigens that do not require microwave retrieval or enzymatic digestion, skip Step 3 in Protocol A.

D. Staining for Blood Smears, Cell Smears and Frozen Sections

1. Use poly-L-lysine-coated adhesive slides. Prepare blood smears from anticoagulated blood by density-gradient centrifugation; use cultured cell smears or adherent cell monolayers. Air-dry frozen sections with fan at room temperature.
2. Fix specimens, preferably with 4 % paraformaldehyde or 10 % formalin for 60-90 min.

3. Prepare blocking solution: mix 1 volume of 30 % H_2O_2 with 50 volumes of pure methanol. Immerse slides at room temperature for 30 min to inactivate endogenous peroxidase. Rinse with distilled water 1-2 times.
4. Follow Steps 5-9 described in Protocol A for subsequent staining.
5. If unsatisfactory results are obtained from direct staining of frozen sections, perform heat-induced antigen retrieval as described in Steps 3-9 of Protocol A.

Important Notes

1. In case of high staining background: after SABC incubation and before DAB development, wash slides 4 times with PBS (pH 7.2-7.6) containing 0.01-0.02 % Tween-20, then wash twice with plain PBS before DAB chromogenic reaction.
2. EDTA antigen retrieval buffer is the preferred choice for heat-induced antigen retrieval.