Product Manual

Rabbit IgG SABC Kit (Peroxidase)

Catalog No.: abx090693 **Revision date:** 30-Apr-20

Size: 1 Kit

Storage: Store all components at 2-8 °C for up to one year.

Introduction

Rabbit IgG SABC Kit (Peroxidase) is designed for use with Rabbit primary antibodies in IHC and ICC. This kit contains a biotin-conjugated Goat anti-Rabbit secondary antibody and peroxidase-conjugated streptavidin. A streptavidin-biotin complex (SABC) is formed since streptavidin has high affinity for biotin. Since streptavidin exhibits very low non-specific binding to tissues and cells, due to its nearly neutral isoelectric point, this kit can be used for low background immunohistochemical analysis. This kit also has high sensitivity due to the large number of peroxidase and streptavidin molecules used.

Kit components

1. 5% BSA: 12 ml
2. Goat anti-Rabbit Antibody (Biotin): 12 ml
3. Streptavidin (Peroxidase): 12 ml

Material required but not provided

1. APES or Poly-L-Lysine
2. 0.02 M PBS (pH 7.2-7.6): dissolve 8.5 g NaCl, 2.8 g anhydrous Na_2HPO_4 and 0.4 g anhydrous NaH_2HPO_4 in 1 L of distilled water. (If hydrous phosphates are used, adjust the weight accordingly)
3. 0.01 M Citrate Buffer: dissolve 3 g sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and 0.4 g citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)
4. DAB Horseradish Peroxidase Chromogenic Kit (abx090660)
5. 0.1% Trypsinase, or other compound digest solution

Immunohistochemistry staining options

The optimal method will depend on the antigen, the tissue, the fixation method and/or primary antibody, and several experiments may be required to determine the best method to use for any given antigen.

- **Heat-induced antigen retrieval.** For exposing binding epitopes on antigens analyzed by IHC-P.
- **Enzymatic antigen retrieval.** For exposing binding epitopes on antigens analyzed by IHC-P.
- **No antigen retrieval.** For stable antigens with non-masked epitopes analyzed by IHC-P.

- **Blood samples, cultured cells and frozen sections.** For blood samples and cultured cells analyzed by ICC, and samples analyzed by IHC-F.

IHC-P assay procedure

1. Cover the entire surface of a clean microscope slide with APES or Poly-L-Lysine. Incubate for 1 min, then rinse the slide with water. Mount a tissue section (~5 µm thick) with the treated slide and heat at 58-60 °C for 30-60 min to ensure strong adhesion of the tissue section.
2. Dewax the tissue section in dimethylbenzene for 10 min and rinse with water.
3. Incubate the tissue section for 5-10 min in 3% H_2O_2 solution to quench any endogenous peroxidase activity. Wash the tissue section with distilled water 3 times, 2 min each time.
4. **Heat-induced antigen retrieval:** Repair the antigen by heat, soak the tissue section in 0.01 M citrate buffer (pH 6.0), and heat to the boiling point with an electric heater or a microwave oven, then stop heating. Allow to cool to room temperature, then wash the tissue section with 0.02 M PBS (pH 7.2-7.6). Repeat this process 1-2 times, leaving a 5-10 min interval between each repeat.
   Enzymatic antigen retrieval: Incubate the tissue section in 0.1% trypsinase or compound digestive solution for 5-10 min. Wash with distilled water 3 times.
   No antigen retrieval: Skip this step.
5. Add 5% BSA blocking reagent solution to the tissue section and incubate at room temperature for 20 min. Discard the blocking reagent solution, but do not wash the tissue section.
6. Add properly diluted primary antibody (Rabbit IgG) to the tissue section and incubate at 37 °C for about 2 hours or at 4 °C overnight. Wash the tissue section with 0.02 M PBS 3 times, 2 min each time. **Note:** 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 biotin-conjugated Goat anti-Rabbit IgG to the tissue section and incubate at 20-37 °C for 20 min. Wash the tissue section with 0.02 M PBS 3 times, 2 min each time.
8. Add SABC-Peroxidase to the tissue section and incubate at 20-37 °C for 20 min. Wash the tissue section 4 times with 0.02 M PBS, 5 min each time.
9. Use DAB Horseradish Peroxidase Chromogenic kit (abx090660) to stain the tissue section. Add 50 µl of Reagent A, 50 µl of Reagent B and 50 µl of Reagent C into 1 ml of distilled water. Mix thoroughly. Add this mixed solution on to the sample and allow the color to develop for 10-30 minutes at room temperature. Color development can be observed under a microscope. Wash the tissue section with distilled water to stop the reaction.
10. Slightly counterstain the tissue section with haematoxylin or Nuclear Fast Red, and wash with distilled water to remove excess haematoxylin. Dry the tissue section by heating, then seal the tissue section with a cover slide. The tissue section is ready for observation under a microscope.

Blood samples, cultured cells and IHC-F assay procedure

1. Cover the entire surface of a clean microscope slide with APES or Poly-L-Lysine. Incubate for 1 min, then rinse the slide with water. Mount a tissue section (~5 µm thick) with the treated slide and heat at 58-60 °C for 30-60 min to ensure strong adhesion of the tissue section.
2. **Blood samples:** Add anticoagulant to the samples and apply the blood samples onto the treated slide.
   Cultured cells: Cultured cells can be added on to the treated slide, or can be directly cultivated on the treated slide.
   Frozen tissue sections: Apply frozen tissue sections to the treated slide, then air-dry at room temperature for 30 min, or until no liquid water is visible.
3. Fix the sample with 4% paraformaldehyde or 10% formalin for 60-90 min.
4. Dilute 30% H_2O_2 at 1/50 with pure methanol. Incubate the fixed sample for 30 min in diluted H_2O_2 to quench any endogenous peroxidase activity. Wash the sample with distilled water 1-2 times. If the direct staining result of frozen tissue sections is not satisfactory, the tissue sections may be repaired by heat-induced antigen retrieval (see Step 4 of the IHC-P assay procedure).
5. Add 5% BSA blocking reagent solution to the tissue section and incubate at room temperature for 20 min. Discard the blocking reagent solution, but do not wash the tissue section.
6. Add properly diluted primary antibody (Rabbit IgG) to the tissue section and incubate at 37 °C for about 2 hours or at 4 °C overnight. Wash the tissue section with 0.02 M PBS 3 times, 2 min each time. **Note:** 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 biotin-conjugated Goat anti-Rabbit IgG to the tissue section and incubate at 20-37 °C for 20 min. Wash the tissue section with 0.02 M PBS 3 times, 2 min each time.
8. Add SABC-Peroxidase to the tissue section and incubate at 20-37 °C for 20 min. Wash the tissue section 4 times with 0.02 M PBS, 5 min each time.
9. Use DAB Horseradish Peroxidase Chromogenic kit (abx090660) to stain the tissue section. Add 50 µl of Reagent A, 50 µl of Reagent B and 50 µl of Reagent C into 1 ml of distilled water. Mix thoroughly. Add this mixed solution on to the sample and allow the color to develop for 10-30 minutes at room temperature. Color development can be observed under a microscope. Wash the tissue section with distilled water to stop the reaction.
10. Slightly counterstain the tissue section with haematoxylin or Nuclear Fast Red, and wash with distilled water to remove excess haematoxylin. Dry the tissue section by heating, then seal the tissue section with a cover slide. The tissue section is ready for observation under a microscope.

Notes

- If the staining background is too high, wash the section 4 times in PBS (pH 7.2-7.6) containing 0.01-0.02% Tween 20, followed by washing 2 times with pure PBS after the SABC reaction and before DAB staining, then use DAB chromogenic kit to stain the section.
- 0.01 M citrate buffer (pH 6.0), PBS, or TBS buffer may be used to repair antigen sections in heat-induced antigen retrieval.