FineTest SABC Kit

Catalogue No.: IHC0001

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING INTRODUCTION

Streptavidin is a 60,000 molecular weight with an extraordinarily high affinity for the small molecular weight vitamin, biotin. Because this affinity is over one million times higher than that of antibody for most antigens, the binding of Streptavidin to biotin (unlike antibody-antigen interactions) is essentially irreversible. In addition to this high affinity, the Biotin/ Streptavidin System can be effectively exploited because Streptavidin has four binding sites for biotin and most proteins (including antibodies and enzymes) can be conjugated with several molecules of biotin. These aspects provide the potential for macromolecular complexes to be formed between Streptavidin and biotinylated enzymes.

An immunoperoxidase procedure based on these properties was devised for localizing a variety of histologically significant antigens and other markers. (Hsu SM, Raine L, Fanger H: Am. J. Clin. Pathol. 7 5, 734-738, 1981; Hsu SM, Raine L, Fanger H: J . Histochem. Cytochem. 2 9, 577-580, 1981.) This technique employs unlabeled primary antibody, followed by biotinylated secondary antibody and then a preformed Avidin and Biotinylated horseradish peroxidase macromolecular Complex.

Finetest SABC kit contains Streptavidin and biotinylated horseradish peroxidase reagents, which have been specially prepared to form ideal complexes for immunoperoxidase staining. Although the structure of the Streptavidin: biotinylated horseradish peroxidase complex is still undefined, evidence suggests that it consists of many biotinylated horseradish peroxidase molecules crosslinked by Streptavidin into a three dimensional array. The complex apparently has few exposed biotin residues but retains at least one biotin binding site. Formation of the complex is achieved by mixing Streptavidin and biotinylated horseradish peroxidase in dilute solution and in defined amounts prior to use. After the initial incubation there appears to be little change in the complex as judged by only a marginal increase in immune peroxidase staining sensitivity and the complex remains stable for several hours after formation. The high sensitivity and shorter incubation times reported for the Finetest SABC system are likely due to the number of active horseradish peroxidase molecules associated with the complex and the rapid, irreversible interaction of the complex with biotinylated antibody. The low background staining

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obtainable with the FineTest SABC Kits is probably due to the high dilutions of primary antisera and other reagents employed in the method, the quality of our affinity-purified biotinylated secondary antibodies, and the specially prepared Streptavidin and biotinylated horseradish peroxidase.

Reagents supplied (for 250 sections)

Blocking Serum	20ml*2	Store at 4°C for 1year
Biotinylated, Affinity-purified **Anti-Rabbit IgG**	500ul	Store at 4°C for 1year
Reagent A (Streptavidin)	500ul	Store at 4°C for 1year
Reagent B (Biotinylated Horseradish Peroxidase)	500ul	Store at 4°C for 1year

NOTE: reagents not supplied (Primary Antibody, Buffer, Hydrogen Peroxide, Oxidizable Peroxidase Substrate)

PREPARATION OF FineTest WORKING SOLUTIONS

A number of different buffers can be used in the FineTest SABC system. One of the most common is 10 mM sodium phosphate, pH 7.4, 0.9% saline (PBS). The SABC working solutions are prepared as follows:

Blocking Serum working solution: add 2ml of stock Blocking Serum to 10 ml of PBS. The preferred serum for blocking is prepared from the same species in which the biotinylated secondary antibody is made.

Biotinylated Antibody working solution: add 100ul Biotinylated Antibody to 10 ml of Blocking Serum working solution (PBS contain Blocking Serum).

SABC Reagent working solution: Mix 100ul of Reagent A and 100ul Reagent B in a EP tube, immediately. Then place it on 37°C for 30 Minutes, add the mixture into 10 ml of Blocking Serum working solution(PBS contain Blocking Serum)for using. It is best to use SABC Reagent working solution within 12 hours.

The configuration table

	Blocking Serum Working solution	Biotinylated Antibody Working solution	SABC Reagent Working solution
1 section	200ul-400ul	200ul	200ul
10 sections	2ml-4ml	2ml	2ml
100 sections	20ml-40ml	20ml	20ml
250 sections	50ml-100ml	50ml	50ml

STAINING PROCEDURE FOR PARAFFIN SECTIONS

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1. Deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.

2. Rinse for 5 minutes in tap water.

3. If quenching of endogenous peroxidase activity is required, incubate the sections for 30 minutes in 0.3% H2O2 in methanol or water. Incubation times may be shortened by using higher concentrations of H2O2. If endogenous peroxidase activity does not present a problem, step 3 may be deleted.

4. Wash in buffer for 5 minutes.

5. Incubate sections for 60 minutes with diluted **Blocking Serum working solution** from the species in which the secondary antibody is made. (In cases where non-specific staining is not a problem, Steps 5 and 6 may be deleted).

6. Blot excess Blocking Serum working solution from sections.

7. Incubate sections for 60 minutes with primary antiserum diluted in buffer. (If background staining occurs, dilutions of the primary and secondary antibodies may be made in buffer containing 1-2% of the appropriate blocking serum.)

8. Wash slides for 5 minutes in buffer.

9. Incubate sections for 60 minutes with diluted biotinylated secondary antibody solution.

10. Wash slides for 5 minutes in buffer.

11. Incubate sections for 30 minutes with FineTest SABC Reagent working solution

12. Wash slides for 5 minutes in buffer.

13. Incubate sections in peroxidase substrate solution until desired stain intensity develops. (See Note 1)

14. Rinse sections in tap water.

15. Counterstain, clear and mount.

NOTES

1. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in diluting the peroxidase substrate or the SABC Reagent. Do not add normal serum, non-fat dried milk, culture media, or other potential sources of biotin to the SABC reagent. This may result in reduced sensitivity.

2. Development times may differ depending upon the level of antigen, the intensity of the stain that is required, or the substrate used. DAB generally should be developed for 2-10 minutes; AEC for 10-30 minutes; TMB for 5-20 minutes. Some counterstains may not be compatible with certain peroxidase substrates because of solubility of the reaction products or lack of color contrast. A counterstain compatibility chart is available upon request.

3. If the reagents are to be diluted beyond their recommended concentrations, first prepare the diluted biotinylated antibody and SABC reagent as described in the instructions. Subsequent dilutions should be made in a buffer containing 0.1% immunohistochemical grade bovine serum albumin. Only immunohistochemical grade BSA

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should be used, as other preparations can containundesired impurities. Dilution of these reagents may require longer incubation times and/or higher incubation temperatures to achieve maximum sensitivities.

4. The section should be well prepared. Fixation (generally, in buffered formalin not exceeding 4 percent formaldehyde) should be sufficient to maintain the integrity of the section throughout the staining procedure but not so harsh as to destroy the antigen under study. During the staining procedure, do not allow the section to dry out. Use a humidified chamber for incubations.

5. To avoid adsorption of the antibody to the plastic or glass container in which the final dilution is made, the primary antibody may be diluted in buffers containing 0.1% immunohistochemical grade bovine serum albumin or dilute Blocking Serum.

6. Incubation times may be shortened. In cases where the antigen concentration in the section is high, suggested incubation times with primary antibody, biotinylated secondary antibody, and SABC Reagent may be reduced. Incubation times as short as five minutes have been reported to be sufficient in some cases when incubation temperatures are raised to 37 °C. If the antigen concentration is low, steps 7 and 9 may be lengthened to achieve maximal staining.

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