Mouse-DAB(Poly-HRP) Detection IHC Kit

Catalogue No.:IHC0008

Size: 200 sections

Storage: 2-8° C for one year, except for Reagent A at -20℃.

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING INTRODUCTION

Fine Biotech adopts a new polymerase-mark system (poly- HRP conjugate Goat Anti-Mouse IgG). More enzymes will connect to the single antibody through the arm structure. Meanwhile, the optimal structure of new system enhences the signal during immunochemistry and reduce the experimental steps.

Reagents supplied

Blocking Serum	20ml	**Store at 2-8°C for 1year**
poly- HRP-Goat Anti-Mouse IgG	15ml	**Store at 2-8°C for 1year**
Reagent A20×concentrated DAB solution	1ml	-20℃
Reagent B20×concentrated solution	1ml	2-8℃
DAB substrate	20ml	2-8℃

NOTE: Reagents not supplied (Primary Antibody, Antibody diluents buffer,) Reagent A (DAB) is a suspected carcinogen. Reagent B contains hydrogen peroxide, handle with care.

PREPARATION OF WORKING SOLUTIONS

A number of different buffers can be used in the IHC system. One of the most common is 10 mM sodium phosphate, pH 7.4, 0.9% saline (PBS). PBS-T (PBS+0.1%Tween 20)

Primary Antibody working solution: according to the dilution ratio provided by the manufacturer.

Blocking Serum and poly- HRP Goat Anti-Mouse IgG: ready-to-use

DAB working solution (1ml): Mix50ul Reagent A, 50ul Reagent Band 900ul DAB substrate in a EP tube. For using, it is best to use DAB working solution within 20minutes.

The configuration table

	Blocking Serum Working solution	poly-HRP-GoatAnti-Mouse IgG	DAB Working solution
1 section	50ul-100ul	70ul	100ul
10 sections	0.5ml-1ml	0.7ml	1ml
100 sections	5ml-10ml	7ml	10ml
200 sections	10ml-20ml	14ml	20ml

STAINING PROCEDURE FOR PARAFFIN SECTIONS

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 1hour at 37℃with **Blocking Serum**

6. Blot excess Blocking Serum from sections.

7. Incubate sections for 1 hourat 37℃or Overnight at 4℃with primary antibody diluted in PBS buffer. (If background staining occurs, dilutions of the primary antibody may be made in buffer containing 0.1% of BSA, See Note 3, 5)

8. Wash slides for 3 minutes in PBST buffer.

9. Incubate sections for 1 hour at 37℃ with **poly- HRP Goat Anti-Mouse IgG**.

10. Wash slides for 3 minutes in PBST buffer.

11. Incubate sections in **DAB working solution** until desired stain intensity develops. (See Note 2)

12. Rinse sections in tap water.

13. Counterstain, clear and mount.

NOTES

1. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used

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. Only immunohistochemical grade BSA 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.

6. Incubation times may be shortened. In cases where the antigen concentration in the section is high, suggested incubation times with primary antibody, secondary antibody, and DAB Reagent may be reduced. If the antigen concentration is low, steps 7 and 9 may be lengthened to achieve maximal staining