1. INTRODUCTION

NICHIREI BIOSCIENCES developed a unique immunohistochemical staining system called Universal Immuno-enzyme Polymer (UIP) method (US Patent 5,846,722). This is NICHIREI BIOSCIENCES’s original technique. Histofine® Simple Stain AP (R), this provides both high sensitivity and time saving in immunohistochemical applications.

2. PRESENTATION

Liquid. Ready to use.

Histofine® Simple Stain AP (R) (Universal Immuno-Alkaline-Phosphatase Polymer, Anti-Rabbit) is the labeled polymer prepared by combining amino acid polymers with Alkaline Phosphatase and goat anti-rabbit Ig which are reduced to Fab. It is stored in MOPS (3-Morpholino propanesulfonic acid) buffer (pH 6.5) containing stabilizer and antibacterial agent.

3. INTENDED USE FOR RESEARCH USE ONLY.

Histofine® Simple Stain AP (R) is designed to allow immunohistochemical staining using a user-supplied primary antibody. This reagent is basically available for smears and formalin-fixed paraffin-embedded human tissue sections. Regarding to the application for staining of frozen tissue sections, please read 6. STAINING PROCEDURES. Also please contact NICHIREI BIOSCIENCES technical service department concerning this reagent for other specimens.

4. PRINCIPLE

The antigen / antibody / Universal Immuno-Alkaline-Phosphatase Polymer complex can be prepared by allowing the reagent to react with a mouse or rabbit primary antibody bound to the antigen on tissue section. The enzymatic activity of this complex results in a colored deposit, thus staining the antigen site.

5. PRECAUTION WHILE USING OR HANDLING.

1. Before using this reagent, please read these instructions. Do not use reagent until after the expiration date.
2. Specimens, before and after fixation, and all other materials exposed to them, should be handled like biotraumatic materials with proper precautions.
3. Inhalation or ingestion of the highly allergic fixative formaldehyde is harmful. Wear protective mask. If swallowed, induce vomiting. If skin or eye contact occurs, wash thoroughly with water.
4. Organic reagents are flammable. Do not use near open flame.
5. Never pipette reagents by mouth and avoid their contact with skin, mucous membranes and clothes.
6. Avoid microbial contamination of reagents as incorrect result may occur.
7. Avoid splashing of reagents or generation of aerosols.
8. For research use only. Not for diagnostic use.

6. STAINING PROCEDURES

1. Addition and reaction of the primary antibody

(1) Wipe areas around the sections on the slides carefully.
(2) Apply 2 drops of primary antibody to specimen slide, positive control slide and negative control slide respectively so as to provide a complete cover of the sections.
(3) To the reagent control slide, apply two drops of negative control reagent (normal serum) in place of primary antibody.
(4) Incubate them at room temperature or 4°C. (Follow the instructions for incubation time data designated in the package insert of primary antibody)
(5) Rinse them in fresh PBS for 3 times, each of 5 minutes duration.
(6) Incubate at room temperature or 4°C. (Follow the instructions for incubation time data designated in the package insert of primary antibody)
(7) Rinse them in fresh PBS for 3 times, each of 5 minutes duration.
(8) Rinse them in TBS for 5 minutes.

2. Addition and reaction of Histofine® Simple Stain AP (R) (Universal Immuno-peroxidase Polymer, Anti-Rabbit)

(1) Wipe areas around the sections on the slides carefully.
(2) Apply 2 drops of Simple Stain AP (MULTI) to each slide so as to provide a complete cover of the sections. Incubate at room temperature for 30 minutes.
(3) Rinse them in fresh PBS for 3 times, each of 5 minutes duration.
(4) Rinse them in TBS for 5 minutes.

3. Addition and reaction of chromogen/substrate solution

(1) Wipe areas around the sections on the slides carefully.
(2) Apply 2 drops of the chromogen/substrate solution to each slide so as to provide a complete cover of the sections. Incubate at room temperature for 5-20 minutes.
(3) Rinse them in distilled water for 3 times, each of 5 minutes duration.

4. Counter-staining

(1) Immerse them in the counterstain solution.
(2) Wash them well with tap water.

5. Mounting

In the case of New Fuchsin substrate, the tissue sections are mounted with water-soluble mounting media or air-dried, cleared in xylene for a few seconds and mounted with permanent mounting media.

[Simple Stain AP (R) Immunohistochemical staining reagent Store at 2-8°C]
8. LIMITATION

Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, or sectioning may produce artifacts or false-negative results.

Results will not be optimal if old or unbuffered fixatives are used, or excessively heated during embedding or during attachment of sections to slides.

False-positive results may be seen due to nonspecific binding of proteins. Although Histofine® Simple Stain AP (R) does not require the use of blocking reagent separately, in some cases the application of blocking reagent containing an irrelevant protein, prior to incubation with the primary antibody, may be useful for reducing the background.

9. CONDITION FOR USE

Histofine® Simple Stain AP (R) is designed for research use only and is not intended for therapeutic or diagnostic purposes. Neither the sales agents and distributors of Histofine® Simple Stain AP (R) nor NICHIREI BIOSCIENCES nor its sales agents can be held responsible for any patent infringement which may occur as a result of improper use of the product.

10. TROUBLE SHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible cause</th>
<th>Solution</th>
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<tbody>
<tr>
<td>No staining or only weak staining results on the positive control slide and the unknown specimen slide</td>
<td>1. Drying out of specimens during staining prior to addition of the reagents.</td>
<td>1. Never allow the tissue to dry out.</td>
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<tr>
<td>The unknown specimen slide is not stained while the positive control slide is stained.</td>
<td>1. Antigen is denatured or masked during fixing or embedding process.</td>
<td>1. Some antigens are sensitive to fixation or embedding. So use less potent fixative and decrease the fixing time.</td>
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<td>The backgrounds are intensively stained in all the slides.</td>
<td>1. Endogenous enzyme activity was not completely blocked.</td>
<td>1. Endogenous enzyme activity was not completely blocked.</td>
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<tr>
<td>During the reaction, tissue sections come off from the slides.</td>
<td>1. Some antigens require heat induced antigen retrieval procedure or prolonged reaction time with primary antibody, which may render the sections easily come off.</td>
<td>1. Add Levamisole to chromogen/substrate solution. To reduce endogenous enzyme activity chromogen/substrate solution containing 1mM Levamisole is used.</td>
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11. REFERENCE


