December, 2012

N-Histofine® High Stain™ HRP (MULTI)

Anti-Mouse and -Rabbit
Immunohistochemical staining reagents

Store at 2 to 8°C

Reagents supplied

	Code: 414481F [170 tests]	Code: 414483F [1000 tests]
High Stain™ Solution A:	17 ml×1 bottle	17 ml×6 bottles
High Stain™ Solution B:	17 ml×1 bottle	17 ml×6 bottles

I. INTRODUCTION

N-Histofine® High Stain™ HRP (MULTI) is a detection system to IHC staining providing enhanced staining performance adopted Universal Immuno-enzyme Polymer (UIP) method (US. Patent No. 6,252,053) developed by Nichirei Biosciences Inc.

II. DESCRIPTION

N-Histofine® High Stain™ HRP (MULTI) is applicable to detection of mouse primary antibody and rabbit primary antibody for human tissue sections.

High Stain™ Solution A

Liquid.
Secondary antibodies of goat anti-mouse Ig and goat anti-rabbit Ig.
Stabilized protein and antibacterial agent are contained.
Ready-to-use solution concentration adjusted with pH 6.5 of MOPS (3-Morpholinopropanesulfonic acid) buffer.

High Stain™ Solution B

Liquid.
Labeled polymer combining amino acid polymers with peroxidase and rabbit anti-goat Ig reduced to Fab'.
Stabilized protein and antibacterial agent are contained.
Ready-to-use solution concentration adjusted with pH 6.5 of MOPS (3-Morpholinopropanesulfonic acid) buffer.

Handling

1. Store reagents at 2 to 8 °C.
2. Adjust reagents to room temperature 15 to 25 °C before use.

III. INTENDED USE

For research use only.

IV. PRINCIPLE

Antigens in tissues are detected by Immunoenzimatic technic. Antigens on human tissue sections are reacted with mouse or rabbit primary antibody, subsequently reacted with High Stain™ solution A, in addition reacted with High Stain™ solution B. Accordingly, complex of antigen, antibody, polymer and enzyme is formed, and chromogen/substrate is developed its color by such enzymatic activity of the complex.
This allows the antigenic site is visualized and presence of antigen is identified by light microscope.

V. STAINING PROCEDURES

1. Reagents and Materials required but not provided

- Xylene
- 95% ethanol
- 100% ethanol
- Phosphate buffered saline (PBS) (pH 7.6±0.2)
  - NaCl 7.75 g
  - K_2HPO_4 1.50 g
  - KH_2PO_4 0.20 g
  - distilled water 1L
- 3% solution of hydrogen peroxide in absolute methanol (Add 1 part of 30% hydrogen peroxide to 9 parts of absolute methanol)
- Mouse or rabbit primary antibody
- Negative control reagent
- Chromogen/substrate reagent
- Counter staining solution
- Distilled water
- Humidified chamber for slide incubation
- Light microscope
- Cover slips
- Mounting media
- Timer
- Staining racks or Coplin jars
- Absorbent wipes
- Slides coated with adhesives such as 0.02% poly-L-lysine or silane for tissue sections

2. Specimen preparation

For optimal fixing maintaining tissue morphology and antigen activity, use of tissue sections as fresh and small as possible (approx. 1cm x 1cm x 0.5cm), and of fixatives shown in the table below is recommended.

Fixatives	Fixing time
10% formalin or buffered formalin	24-48 hours
20% formalin	12-24 hours

Do not allow tissue sections to dry out at any point of during staining processes.

3. Preparation of tissue sections and slides

(1) Paraffin embedded tissue sections
Slice tissue section into 3-6 μm and place on slides.
When further treatments of antigen recovery, Heat-Induced Epitope Retrieval (HIER) or enzyme treatment are conducted, use slides coated with adhesives such as 0.02% poly-L-lysine or silane for tissue sections.

(2) Specimen sample slides
Prepare 2 slides per each specimen as specimen sample slides.
One of these as reagent control slide is stained using negative control (mouse normal serum or rabbit normal serum) alternative of primary antibody.

(3) Specimen control slides
Prepare 2 slides per following each control slide by the same preparation method as the specimen sample slides.

- **Positive control slides**
  Tissue section slides, presence of target antigen is confirmed in advance.
- **Negative control slides**
  Tissue section slides, absence of target antigen is confirmed in advance.

Conduct the entire process of staining and microscopic observation of staining results using aforementioned all the slides.

4. Deparaffinization and Rehydration

(1) Treatment with xylene

1) Immerse the slides in xylene for 3 minutes.
2) Remove excess fluid and immerse the slides in fresh xylene for 3 minutes.
3) Remove excess fluid and immerse the slides in another fresh xylene for 3 minutes.

(2) Treatment with ethanol

1) Immerse the slides in 100% ethanol for 3 minutes.
2) Remove excess fluid and immerse the slides in fresh 100% ethanol for 3 minutes.
3) Remove excess fluid and immerse the slides in 95% ethanol for 3 minutes.
4) Remove excess fluid and immerse the slides in fresh 95% ethanol for 3 minutes.

(3) Washing
Remove excess fluid and rinse well with PBS or buffer for 3 minutes each changing container twice or using washing bottle.

5. Staining Procedures

According to primary antibody applied, pretreatment of tissue sections may be required. In that case, such sections should be treated properly referring instruction of the antibody.
When endogenous peroxidase, erythrocytes or granulocytes are not contained in tissue sections, quenching of endogenous peroxidase may be omitted.

(1) Quenching of endogenous peroxidase

1) Wipe around tissue sections on the slides carefully to remove excess solution.
2) Immerse the slides in 3% solution of hydrogen peroxide in absolute methanol for 5-15 minutes at room temperature.
3) Rinse the slides in fresh PBS 3 times for 5 minutes each.

(2) Addition and reaction of the primary antibody

1) Wipe around tissue sections on the slides carefully.
2) Apply 2 drops (100μl) of primary antibody to specimen slide, positive control slide and negative control slide respectively covering the tissue sections completely.
3) To the reagent control slide, apply 2 drops of negative control reagent (normal serum) in place of primary antibody.

4) Incubate the slides at room temperature or storage temperature. (Refer to the instruction of the antibody)
5) Rinse the slides in fresh PBS 3 times for 5 minutes each.

(3) Addition and reaction of High Stain™ Solution A
1) Wipe around tissue sections on the slides carefully.
2) Apply 2 drops (100μl) of High Stain™ Solution A to each slide covering the sections completely. Incubate the slides at room temperature for 10 minutes.
3) Rinse the slides in PBS well.

(4) Addition and reaction of High Stain™ Solution B
1) Wipe around tissue sections on the slides carefully.
2) Apply 2 drops (100μl) of High Stain™ Solution B to each slide covering the sections completely. Incubate the slides at room temperature for 10 minutes.
3) Rinse the slides in PBS well.

(5) Addition and reaction of chromogen/substrate reagent*
1) Wipe around the tissue sections on the slides carefully.
2) Apply 2 drops (100μl) of the chromogen/substrate reagent to each slide covering the sections completely. Incubate the slides at room temperature for 5-20 minutes.
3) Rinse the slides in fresh PBS 3 times for 5 minutes each.

* Handle chromogen/substrate reagents with adequate attention, if 3,3'-diaminobenzidine tetrahydrochloride (DAB), recognized as mutagens, is contained.

(6) Counter-staining
1) Immerse the slides in counterstaining solution.
2) Wash the slides well with tap water.

(7) Mounting
When chromogen/substrate reagent AEC is used, tissue sections are mounted with water-based mounting media without further treatment.
When chromogen/substrate reagent DAB is used, tissue sections are mounted with permanent mounting media after washing with water, dehydrated in graded series of alcohol and cleared in xylene.

6. Interpretation of staining results
Examine the slides under light microscope for staining results.
Interpret the staining results by comparing following three different control slides.

- **Positive control slides**
  Positive staining result is observed.
- **Negative control slides**
  Positive staining result is not observed.
- **Reagent control slides**
  Positive staining result is not observed.
  Non-specific reaction by binding of non-specific proteins as well is considered when this slide is stained.

VI. STORAGE
Store in a dark place at 2-8°C.

VII. PRECAUTIONS
1. Read this instruction before use.
2. Do not use reagents after the expiration date.
3. Do not use reagents mixed with different lots or manufacturers, or as combinations.
4. Handle reagents properly as with biological products due to High Stain Solution A and Solution B contain material derived from animal.
5. Handle human derived specimen with adequate attention as its risk of infection.
6. When the reagents are splattered, wipe up and sterilize such part with alcohol sprayed as well.
7. Avoid inhale reagents or contact with eyes, mouth, skin and clothing as well.
8. Refer to MSDS of this product for further safety information.
9. For professional users.
10. N-Histofine® is a registered trademark of Nichirei Biosciences Inc.

VIII. LIMITATION
1. N -Histofine® High Stain™ HRP (MULTI) is for use on formalin-fixed, paraffin-embedded tissue sections only. When frozen sections or cell smears are used, optimal condition should be verified and determined by users.
2. Optimal concentration and incubation time of primary antibodies should be determined by investigation. In some cases, further dilution of primary antibodies may be required to prevent over staining.
3. Staining results are subject to the condition of entire staining procedures. Improper fixation, freezing, thawing, washing, drying, heating, or sectioning may cause artifacts or false-negative results.
4. Results may not be optimal if fixatives beyond expiration date or unbuffered are used, or slides are overheated during embedding or adhesion of tissue sections.
5. N-Histofine® High Stain™ HRP (MULTI) is designed for research use only and is not intended for therapeutic or diagnostic purposes. NICHIREI BIOSCIENCES INC., its sales agents and distributors will take no responsibility for N-Histofine® High Stain™ HRP (MULTI) when used in a way which directly or indirectly violates local regulations or patents. Neither NICHIREI BIOSCIENCES INC. nor its sales agents can be held responsible for any patent infringement which may occur as a result of improper use of the product.

IX. TROUBLE SHOOTING

Problem	Possible cause	Solution
No staining or weak staining on positive control slide and specimen slide.	1. Specimen is dried out.	1. Prevent tissue section being dried out after moisturized.
	2. Inadequate embedding agent or incomplete deparaffinization from paraffin-embedded tissue sections.	2-1. Apply adequate embedding agent or complete deparaffinization of tissue sections embedded. 2-2.Change xylene or ethanol.
	3. Insufficient reaction of High Stain solution A and solution B.	3. Prolong reaction time of High Stain solution A and solution B for 15 to 20 minutes.
	4. Insufficient reaction of chromogen/substrate reagent.	4. Prolong reaction time of chromogen/substrate reagent.
No staining on specimen slide while positive control slide is stained.	1. Denaturation or masking of antigen during fixing or embedding process.	1. Apply mild fixative and shorten the fixing time due to some antigens are sensitive to fixation or embedding.
	2. Decomposing of antigen by autolysis.	2. Fix freshly obtained tissues by appropriate method.
	3. Low level of antigen presented in tissue sections.	3-1. Prolong reaction time of High Stain solution A and solution B for 15 to 20 minutes. 3-2. Prolong reaction time of chromogen/substrate reagent.
Backgrounds are stained intensively on all the slides.	1. Insufficient inactivation process of endogenous peroxidase.	1. Apply specified processing time, 10 to15 minutes, of blocking reagent (3% of hydrogen peroxide).
	2. Excessive presence of antigens isolated in histological solution by autolysis.	2. Embed fresh tissues whenever available.
	3. Incomplete deparaffinization.	3. Change xylene or ethanol.
	4. Insufficient washing of antibody.	4. Increase washing frequency of antibody.
	5. Too high room temperature accelerates enzyme reactions.	5. Keep room temperature at 15 to 25°C.
Tissue sections are come off from slides during reaction.	1. Insufficient tissue section adhesion on slide.	1. Mount tissue sections on slides coated with adhesive such as 0.02% poly-L-lysine or silane.

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