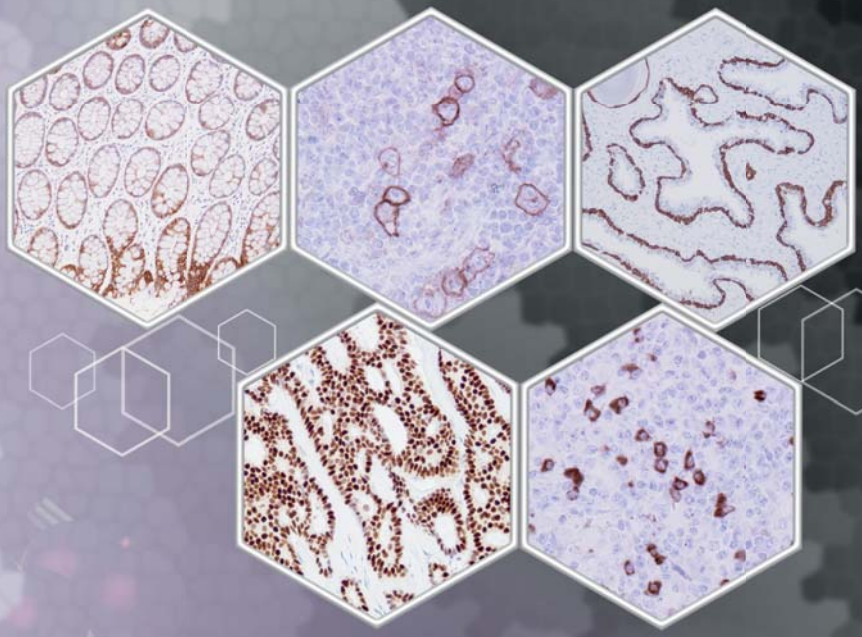
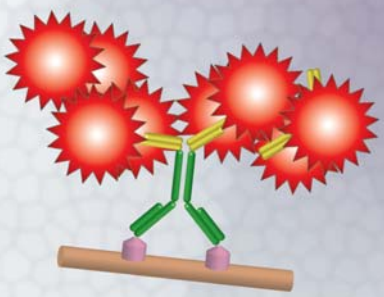
Catalog

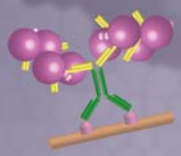
2012-2013

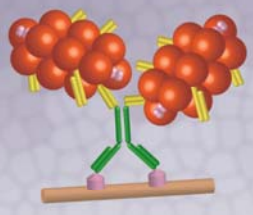


IHC

Immunohistochemistry

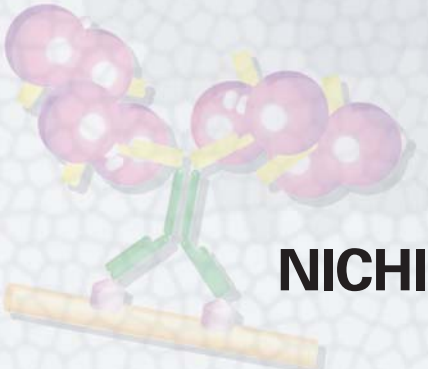






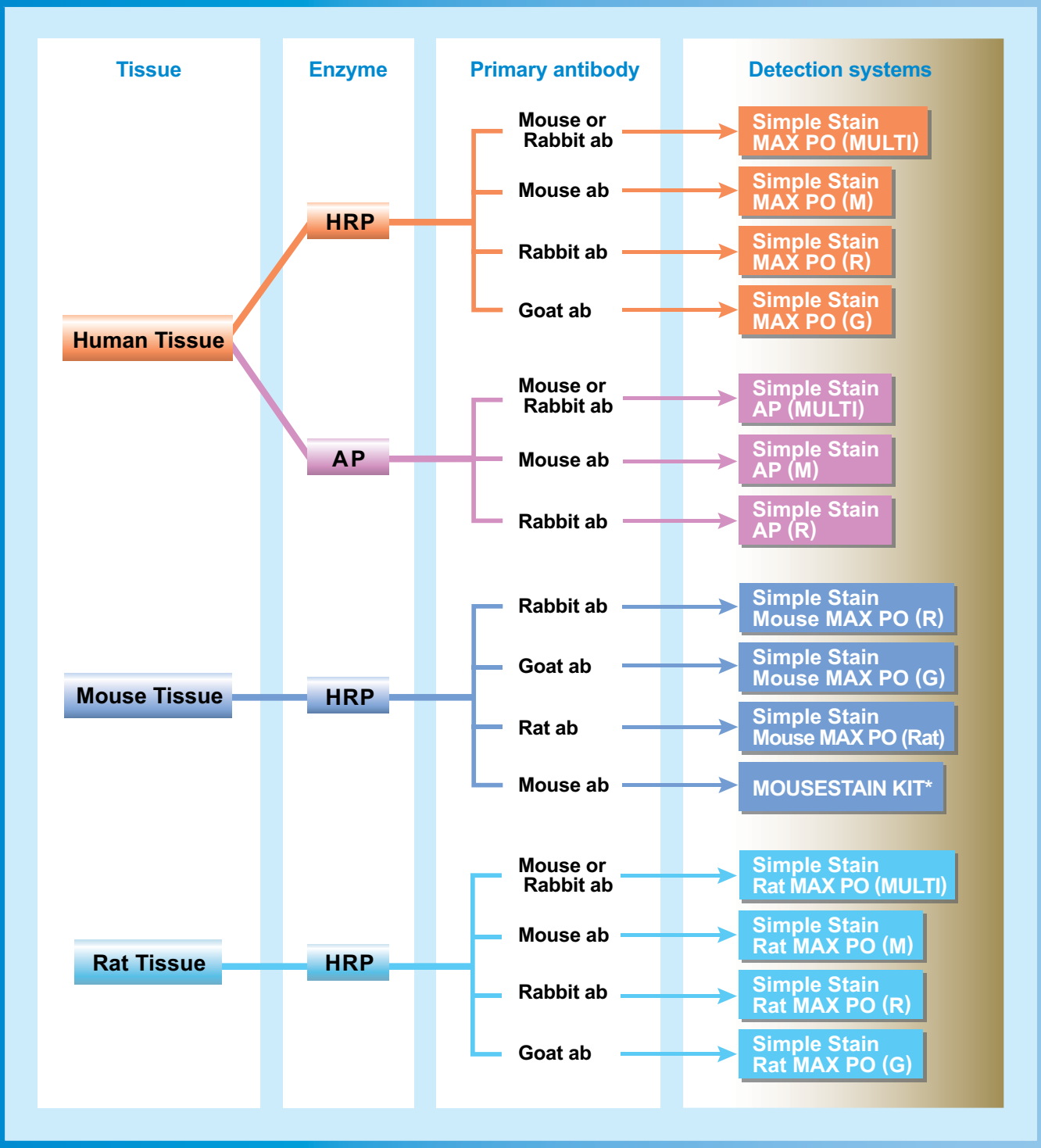
N-Histofine®

Polymer Detection System



NICHIREI BIOSCIENCES INC.

N-Histofine® series application on IHC staining



*: MOUSESTAIN KIT is not available in the United States.

About us

Nichirei Corporation had started bioscience business as a manufacture of Anti-Leukocytes Monoclonal antibodies (CD Antibodies) in 1983.
Nichirei Biosciences Inc. (hereinafter called “Nichirei Bio”) was reorganized on April 1, 2005 succeeding Nichirei’ s bioscience business adopting the holding company system by its parent company, Nichirei Corporation. Nichirei Bio’ s mission is to contribute to the development of the global Bio-industries including medical, cosmetic and healthcare business as a specialized company by offering high-quality products and services based on its advanced technology.

Nichirei Bio’s business field

1. IHC (Immunohistochemistory) products (N-Histofine®)

Nichirei Bio has developed and improved patented state-of-the-art technology, UIP (Universal Immuo-enzyme Polymer) method. Nichirei Bio manufactures and supplies N-Histofine® products including CE marked products adopted the UIP technology. Also for domestic use, Nichirei Bio manufactures and supplies several IVD products for IHC.

2. Diagnostic products (EIA and Lateral Flow Assay kits)

Nichirei Bio develops, manufactures and distributes several IVD products immuno-reaction applied, especially POC (Point of Care) for rapid diagnostics.

3. Cell-biology products

Nichirei Bio provides animal sera and media related to cell culture to the customers both in the academic and industrial field.

4. Functional Materials

Nichirei Bio develops functional materials (powder and extract) from natural raw materials such as Acerola and Camu Camu fruits procured by consolidated subsidiaries and affiliates of the Nichirei group. Nichirei Bio supplies such materials to cosmetics and health food manufacturers.

Contents

What's New

ALK Detection Kit

N-Histofine® ALK Detection Kit detects anaplastic lymphoma kinase (ALK) proteins in tumor cells in paraffin-embedded tissue specimens by IHC staining and determines presence of such protein expression.

This kit is applicable to *low expression of ALK fusion proteins* as well.

For details, see Page 7

Product Name	Tests	Components		Code
N-Histofine® ALK Detection Kit	20	1 Blocking Reagent	4ml×1	417071F
		2 Primary Antibody	2ml×1	
		3 Negative Control	2ml×1	
		4 Bridge Reagent	4ml×1	
		5 Peroxidase Labeled Empower Reagent	4ml×1	
		6 Chromogen Substrate	0.5ml×1	
		7 Substrate Buffer Solution	0.5ml×1	
		8 Chromogen Reagent	0.5ml×1	
		9 ALK Antigen Retrieval Solution A	150ml×1	
		10 ALK Antigen Retrieval Solution B	150ml×1	

ALK Control slides

N-Histofine® ALK Control Slides are as a standatd for expression of ALK proteins and used with N-Histofine® ALK Detection Kit.

Both formalin fixed paraffin embedded cell lines of NCI-H2228 (Positive) and SK-BR-3 (Negative) are mounted on each slide.

For details, see Page 8

Product Name	Slides	Code
N-Histofine® ALK Control Slides	5	417081F

Detection system for Human tissue sections

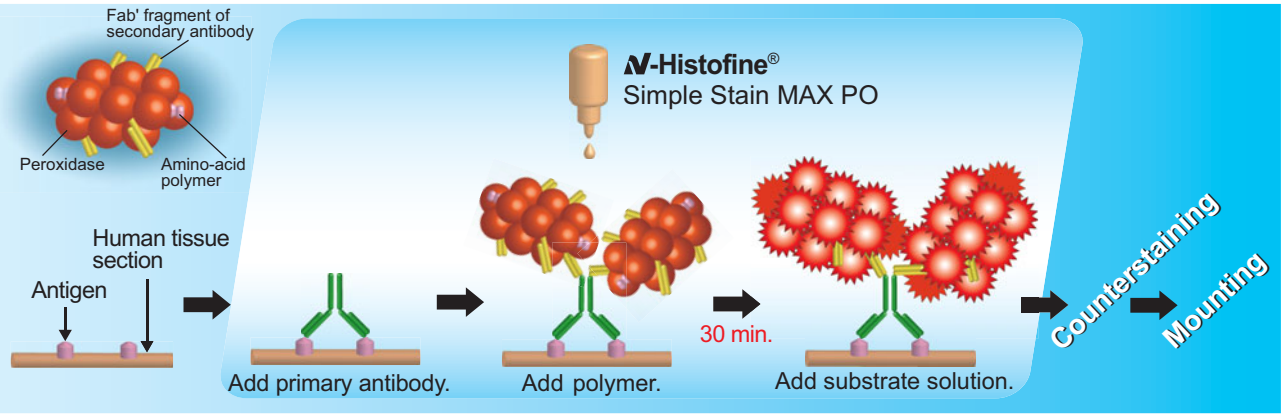
N-Histofine® Simple Stain MAX PO

This product adopted the UIP (Universal Immuno-enzyme Polymer) method, Nichirei Bio's patented technology.

Feature

N-Histofine® Simple Stain MAX PO is a detection reagent designed specifically to allow immunohistochemical staining on formalin-fixed paraffin-embedded human tissue sections. It is the labeled polymer prepared by combining amino acid polymers with multiple molecules of peroxidase (PO) and secondary antibody which is reduced to Fab' fragment. To eliminate background staining, solid-phase absorption of secondary antibody is carried out with human serum.

Principle & Procedure

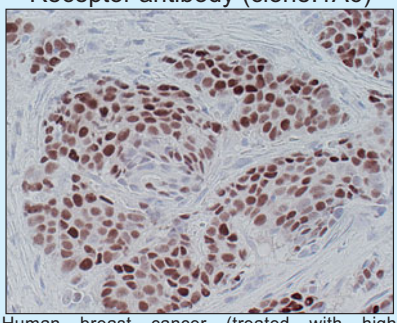


Advantages

1. Simplified staining steps (See page 12).
2. High sensitivity (See page 12).
3. Unaffected by endogenous biotin in the tissue (See page 12).
4. Ready to use.

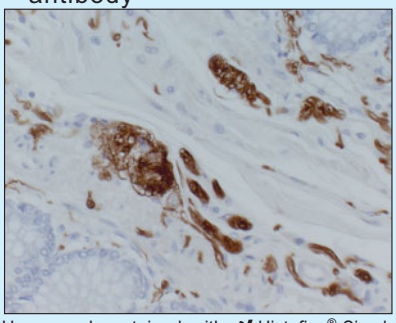
Staining Results

■ Mouse anti-Progesteron Receptor antibody (clone:1A6)



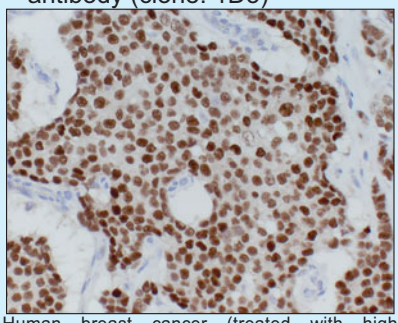
Human breast cancer (treated with high temperature epitope unmasking method) stained with N-Histofine® Simple Stain MAX PO(M) and DAB chromogen.
Note nuclear staining of breast cancer cells.

■Rabbit anti-S-100 protein antibody



Human colon stained with N-Histofine® Simple Stain MAX PO(R) and DAB chromogen.
Note cytoplasmic staining of nerve cells.

■Mouse anti-Estrogen Receptor antibody (clone: 1D5)



Human breast cancer (treated with high temperature epitope unmasking method) stained with N-Histofine® Simple Stain MAX PO (MULTI) and DAB chromogen.
Note nuclear staining of breast cancer cells.

Product

Detection systems.

Liquid. Ready to use.

Product Name	For (slides)	Volume	Code	For use with	Mark in EU
N-Histofine® Simple Stain MAX PO (MULTI)	170	17ml x 1	414151F	Mouse and rabbit primary antibodies	CE
	500	17ml x 3	414152F		
	1500	17ml x 9	414154F		
N-Histofine® Simple Stain MAX PO (M)	170	17ml x 1	414131F	Mouse primary antibody	
	500	17ml x 3	414132F		
	1500	17ml x 9	414134F		
N-Histofine® Simple Stain MAX PO (R)	170	17ml x 1	414141F	Rabbit primary antibody	
	500	17ml x 3	414142F		
	1500	17ml x 9	414144F		
N-Histofine® Simple Stain MAX PO (G)	170	17ml x 1	414161F	Goat primary antibody	
	500	17ml x 3	414162F		

Detection system for Human tissue sections

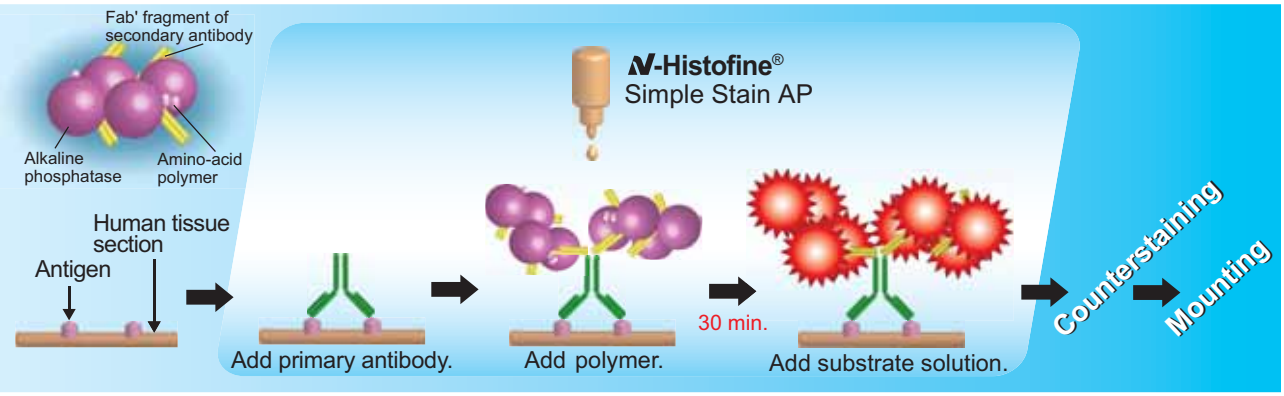
N-Histofine® Simple Stain AP

This product adopted the UIP (Universal Immuno-enzyme Polymer) method, Nichirei Bio's patented technology.

Feature

N-Histofine® Simple Stain AP is a detection reagent designed specifically to allow immunohistochemical staining on formalin-fixed paraffin-embedded human tissue sections. It is the labeled polymer prepared by combining amino acid polymer with alkaline phosphatase (AP) and secondary antibody which is reduced to Fab' fragment. To eliminate background staining, solid-phase absorption of secondary antibody is carried out with human serum.

Principle & Procedure

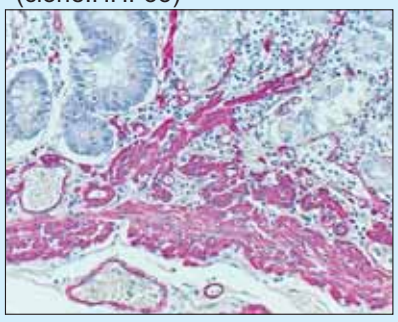


Advantages

1. Simplified staining steps (See page 12).
2. High sensitivity (See page 12).
3. Unaffected by endogenous biotin in the tissue (See page 12).
4. Ready to use.

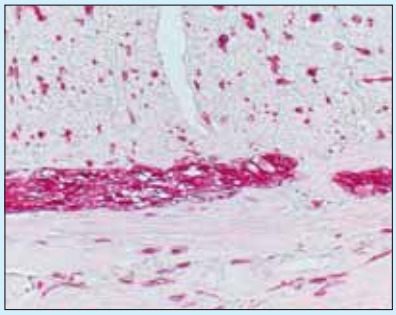
Staining Results

■Mouse anti-Muscle Actin antibody (clone:HHF35)



Human stomach stained with N-Histofine® Simple Stain AP (M) and New fuchsin chromogen.
Note intense staining of smooth muscle in the walls of blood vessel and muscularis mucosae.

■Rabbit anti-S-100 protein antibody



Human colon stained with N-Histofine® Simple Stain AP (R) and New fuchsin chromogen.
Note cyroplasmic staining of nerve cells scattered in smooth muscle and Auerbach's plexus.

Product

Detection systems.

Liquid. Ready to use.

Product Name	For (slides)	Volume	Code	For use with
N-Histofine® Simple Stain AP (MULTI)	170	17ml x 1	414261F	Mouse and rabbit primary antibodies
	500	17ml x 3	414262F	
N-Histofine® Simple Stain AP (M)	170	17ml x 1	414241F	Mouse primary antibody
	500	17ml x 3	414242F	
N-Histofine® Simple Stain AP (R)	170	17ml x 1	414251F	Rabbit primary antibody
	500	17ml x 3	414252F	

Detection system for Human tissue sections

N-Histofine® ALK Detection KIT

For research use only

Feature

N-Histofine® ALK Detection Kit detects anaplastic lymphoma kinase (ALK) proteins in tumor cells in paraffin-embedded tissue specimens by IHC staining and determines presence of such protein expression.

Advantage

This kit is applicable to low expression of ALK fusion proteins as well.

Background

The *ALK* gene was identified in 1994 as a gene fused to the nucleophosmin (*NPM*) gene in anaplastic large-cell lymphoma (ALCL) with t(2;5)(p23;q35) translocation. This gene is located at 2p23 and encodes for a receptor-type tyrosine kinase, which belongs to the insulin receptor family. The ALK protein have a kinase domain in its intracellular domain, and its function is associated with the promotion of cell growth and inhibition of apoptosis.

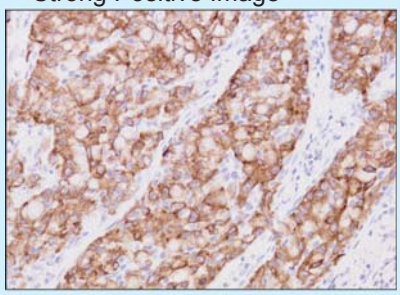
Subsequently, the *ALK* gene has been reported to form *ALK* fusion genes fused with *ATIC, CLTC, MSN, TPM3, TPM4, TFG, MYH9* and *ALO17* genes in ALCL and also *ATIC, CARS, CLTC, DCTN1, TPM3, TPM4, PPFIBP1, RANBP2* and *SEC31L1* genes in inflammatory myofibroblastic tumor (IMT).

The proteins produced from these fusion genes are constantly activated by forming dimers and led to cancerous change.

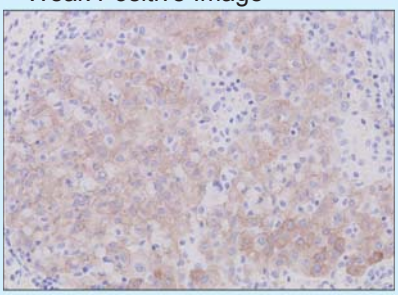
Recently, other *ALK* fusion genes with *EML4* gene, *KIF5B* gene or *KCL1* gene in non-small cell carcinoma of lung, *SEC31A* gene or *SQSTM1* gene in ALK-positive large B-cell lymphoma and *VCL* gene in renal cell cancer have been also reported.

Staining Images

■ Strong Positive Image



■ Weak Positive Image



Lung adenocarcinoma: Positive for cytoplasm of tumor cells. Weak to strong positive of staining levels regarding the expression level of ALK fusion protein. Hot bath treatment (+)

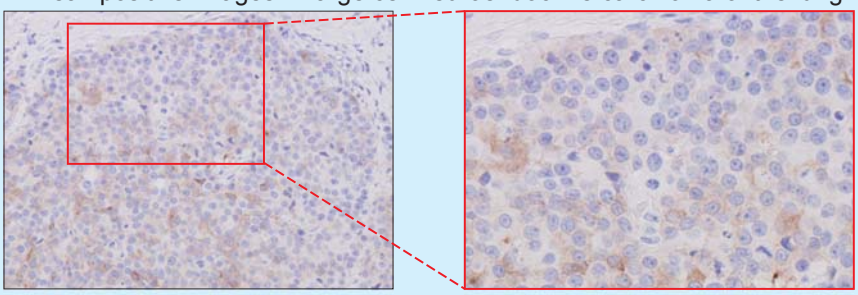
Notes for Determination

Due to this kit detects ALK proteins, ALK fusion proteins as well as full-length ALK protein are reacted. Consequently, results of slight positive to positive for tumors* that infrequently express full-length ALK protein are observed. However, discrimination between ALK fusion proteins and full-length ALK protein is not available. Therefore, considering expression possibility of ALK fusion proteins, confirmation of the presence or absence of *ALK* fusion genes by using FISH method is preferable in this regard.

* Large-cell neuroendocrine carcinomas of the lung, small-cell lung carcinomas and rhabdomyosarcomas (particularly alveolar rhabdomyosarcomas).

Staining Images

■ Weak positive Images in large cell neuroendocrine carcinoma of the lung



Weak positive staining is observed in the specimen which expresses full-length ALK protein.
Hot bath treatment (+)

Staining Flowchart

Deparaffinization and Rehydration
↓
Antigen Unmasking Treatment
↓
Quenching of endogenous peroxidase
↓
Reaction with Primary Antibody or Negative Control
↓
Reaction with Bridge Reagent
↓
Reaction with Peroxidase labeled Empower Reagent
↓
Reaction with chromogen/substrate reagent
↓
Counterstaining
↓
Mounting

Note

High temperature epitope unmasking is required for formalin fixed paraffin-embedded tissue sections prior to IHC staining.

References

(1) Shiota M., et al: Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. Oncogene 9: 1567-1574, 1994

(2) Morris SW., et al: Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science. 263: 1281-1284, 1994

(3) Takeuchi K., et al: KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res. 15(9): 3143-3149, 2009

(4) Takeuchi K., et al: Pulmonary inflammatory myofibroblastic tumor expressing a novel fusion, PPFIBP1-ALK: reappraisal of anti-ALK immunohistochemistry as a tool for novel ALK-fusion identification. Clin Cancer Res. 17(10): 3341-3348, 2011

(5) Sugawara E., et al: Identification of Anaplastic Lymphoma Kinase Fusions in Renal Cancer. Cancer, 2012

N-Histofine® ALK Control Slides

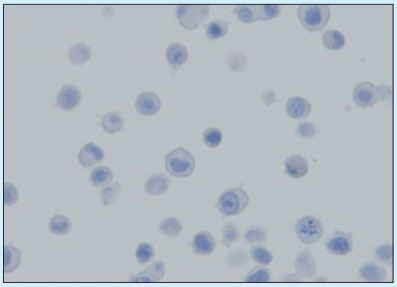
Both formalin fixed paraffin embedded cell lines of NCI-H2228 (Positive) and SK-BR-3 (Negative) are mounted on each slide.

N-Histofine® ALK Control Slides are used as a standard for discrimination of positive tissue blocks possess expressed ALK protein.

This product is used for validation of reagent performance and staining technique in the IHC staining (including cytology staining) with N-Histofine® ALK Detection Kit.

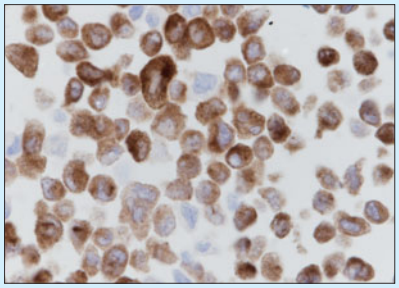
Staining Images

■ **Negative** control cell line/SK-BR-3



No stain is observed in the cytoplasm of all cells. Treatment with hot bath (+)

■ **Positive** control cell line/NCI-H2228



Strong stains are observed in the cytoplasm of the majority of cells. Treatment with hot bath (+)

Product

Detection system.

Liquid. Ready to use.

Product Name	Size	Code
N-Histofine® ALK Detection Kit	20 tests	417071F

Vial No.	Components	Constituents	Volume
1	Blocking Reagent	3 V/V% Hydrogen peroxide	4ml × 1
2	Primary Antibody	anti-ALK mouse monoclonal antibody (5A4)	2ml × 1
3	Negative Control	Mouse IgG	2ml × 1
4	Bridge Reagent		4ml × 1
5	Peroxidase Labeled Empower Reagent		4ml × 1
6	Chromogen Substrate	3,3'-Diaminobenzidine tetrahydrochloride	0.5ml × 1
7	Substrate Buffer Solution		0.5ml × 1
8	Chromogen Reagent	0.6 V/V% Hydrogen peroxide solution	0.5ml × 1
9	ALK Antigen Retrieval Solution A		150ml × 1
10	ALK Antigen Retrieval Solution B		150ml × 1

Product Name	Size	Code
N-Histofine® ALK Control Slides	5 slides	417081F

Detection system for Rat tissue sections

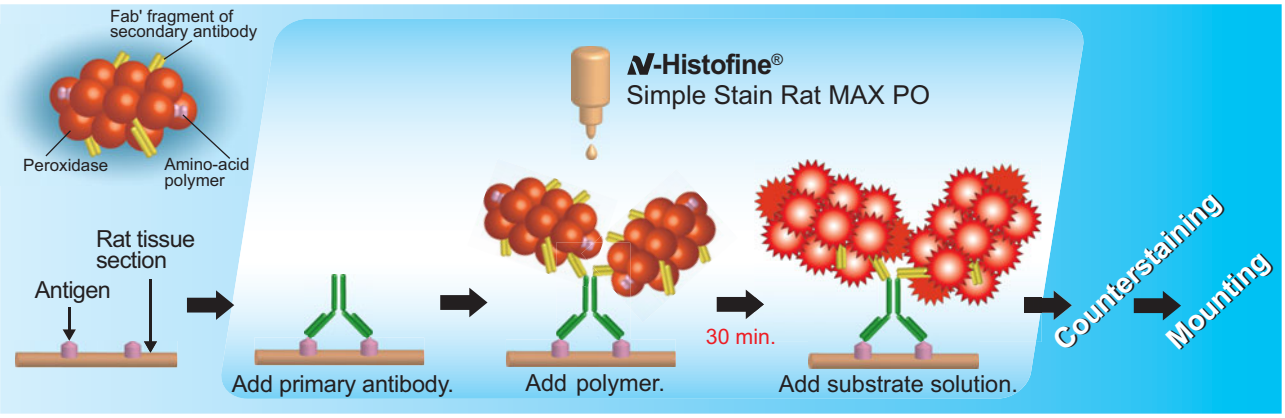
N-Histofine® Simple Stain Rat MAX PO

This product adopted the UIP (Universal Immuno-enzyme Polymer) method, Nichirei Bio's patented technology.

Feature

N-Histofine® Simple Stain Rat MAX PO is a detection reagent designed specifically to allow immunohistochemical staining on formalin-fixed paraffin-embedded rat tissue sections. It is the labeled polymer prepared by combining amino acid polymers with multiple molecules of peroxidase (PO) and secondary antibody which is reduced to Fab' fragment. To eliminate background staining, solid-phase absorption of secondary antibody is carried out with rat, human, dog, pig and bovine sera.

Principle & Procedure

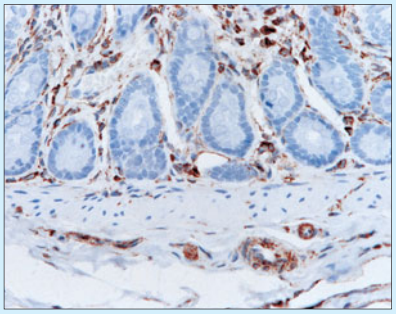


Advantages

1. No reaction to endogenous rat immunoglobulin in the tissue (See page 18).
2. Simplified staining steps (See page 12).
3. High sensitivity (See page 12).
4. Unaffected by endogenous biotin in the tissue (See page 12).
5. Ready to use.

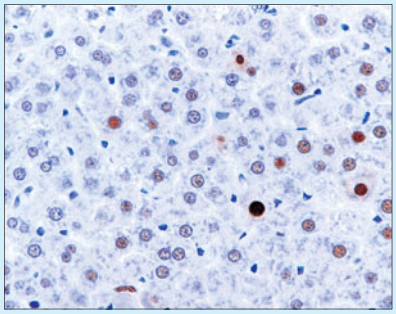
Staining Results

■Mouse anti-Vimentin antibody (clone:V9)



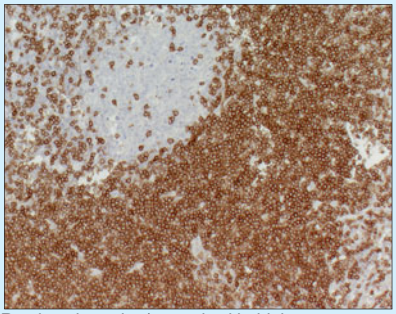
Rat colon (treated with high temperature epitope unmasking method) stained with N-Histofine® Simple Stain Rat MAX PO (M) and DAB chromogen.
Note cytoplasmic staining of fibroblasts and endothelial cells.

■Mouse anti-PCNA antibody (clone:PC10)



Rat liver stained with N-Histofine® Simple Stain Rat MAX PO (MULTI) and DAB chromogen.
Note nuclear staining of liver cells.

■Goat anti-CD3-ε(M-20) antibody



Rat lymph node (treated with high temperature epitope unmasking method) stained with N-Histofine® Simple Stain Rat MAX PO (G) and DAB chromogen.
Note membrane staining of almost all lymphocytes.

Product

Detection systems.

Liquid. Ready to use.

Product Name	For (slides)	Volume	Code	For use with
N-Histofine® Simple Stain Rat MAX PO (MULTI)	170	17ml	414191F	Mouse and rabbit primary antibodies
N-Histofine® Simple Stain Rat MAX PO (M)	170	17ml	414171F	Mouse primary antibody
N-Histofine® Simple Stain Rat MAX PO (R)	170	17ml	414181F	Rabbit primary antibody
N-Histofine® Simple Stain Rat MAX PO (G)	170	17ml	414331F	Goat primary antibody

Detection system for Mouse tissue sections

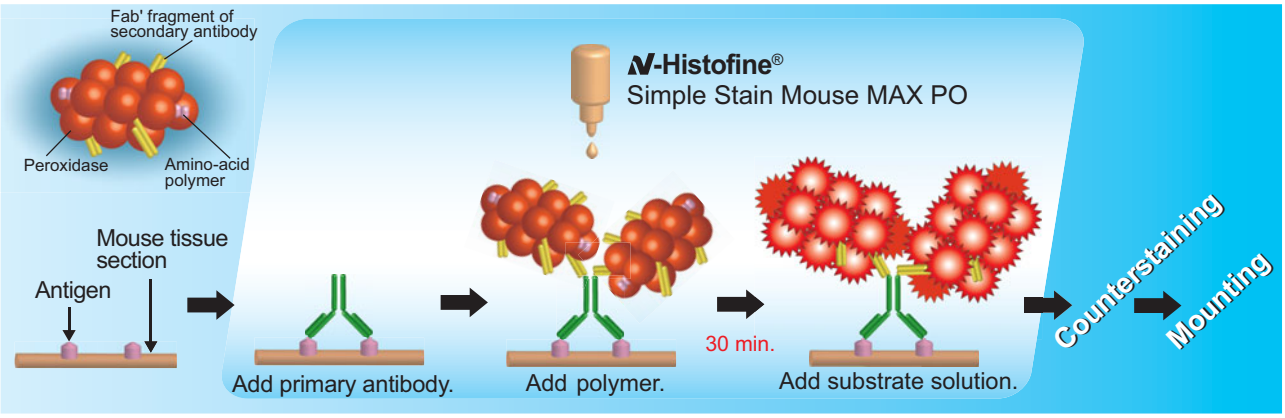
N-Histofine® Simple Stain Mouse MAX PO

This product adopted the UIP (Universal Immuno-enzyme Polymer) method, Nichirei Bio's patented technology.

Feature

N-Histofine® Simple Stain Mouse MAX PO is a detection reagent designed specifically to allow immunohistochemical staining on formalin-fixed paraffin-embedded mouse tissue sections. It is the labeled polymer prepared by combining amino acid polymers with multiple molecules of peroxidase (PO) and secondary antibody which is reduced to Fab' fragment. To eliminate background staining, solid-phase absorption of secondary antibody is carried out with mouse serum.

Principle & Procedure

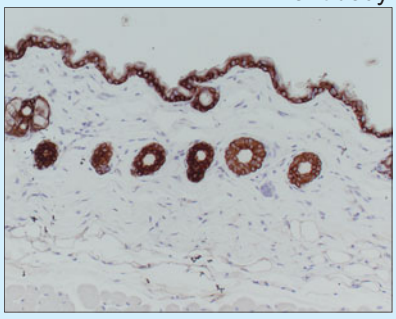


Advantages

1. No reaction to endogenous mouse immunoglobulin in the tissue (See page 18).
2. Simplified staining steps (See page 12).
3. High sensitivity (See page 12).
4. Unaffected by endogenous biotin in the tissue (See page 12).
5. Ready to use.

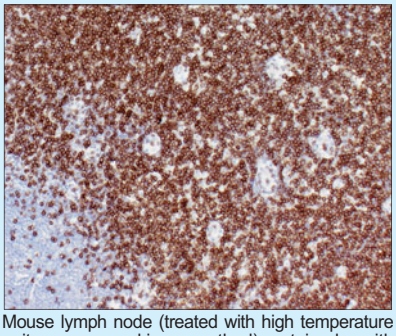
Staining Results

■Rabbit anti-Keratin/Cytokeratin antibody



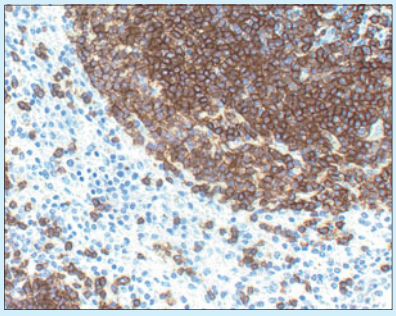
Mouse skin stained with N-Histofine® Simple Stain Mouse MAX PO(R) and DAB chromogen.
Note cytoplasmic staining of epithelium cells and sweat gland cells.

■Goat anti-CD3-ε(M-20) antibody



Mouse lymph node (treated with high temperature epitope unmasking method) stained with N-Histofine® Simple Stain Mouse MAX PO(G) and DAB chromogen.
Note membrane staining of almost all lymphocytes.

■Rat anti-Mouse CD45R/B220



Mouse spleen stained with N-Histofine® Simple Stain Mouse MAX PO (Rat) and DAB chromogen.
Note membrane staining of almost all lymphocytes in germinal center and scattered interfollicular lymphocytes.

Product

Detection systems.

Liquid. Ready to use.

Product Name	For (slides)	Volume	Code	For use with
N-Histofine® Simple Stain Mouse MAX PO (R)	170	17ml	414341F	Rabbit primary antibody
N-Histofine® Simple Stain Mouse MAX PO (G)	170	17ml	414351F	Goat primary antibody
N-Histofine® Simple Stain Mouse MAX PO (Rat)	170	17ml	414311F	Rat primary antibodies

Detection system for Mouse tissue sections

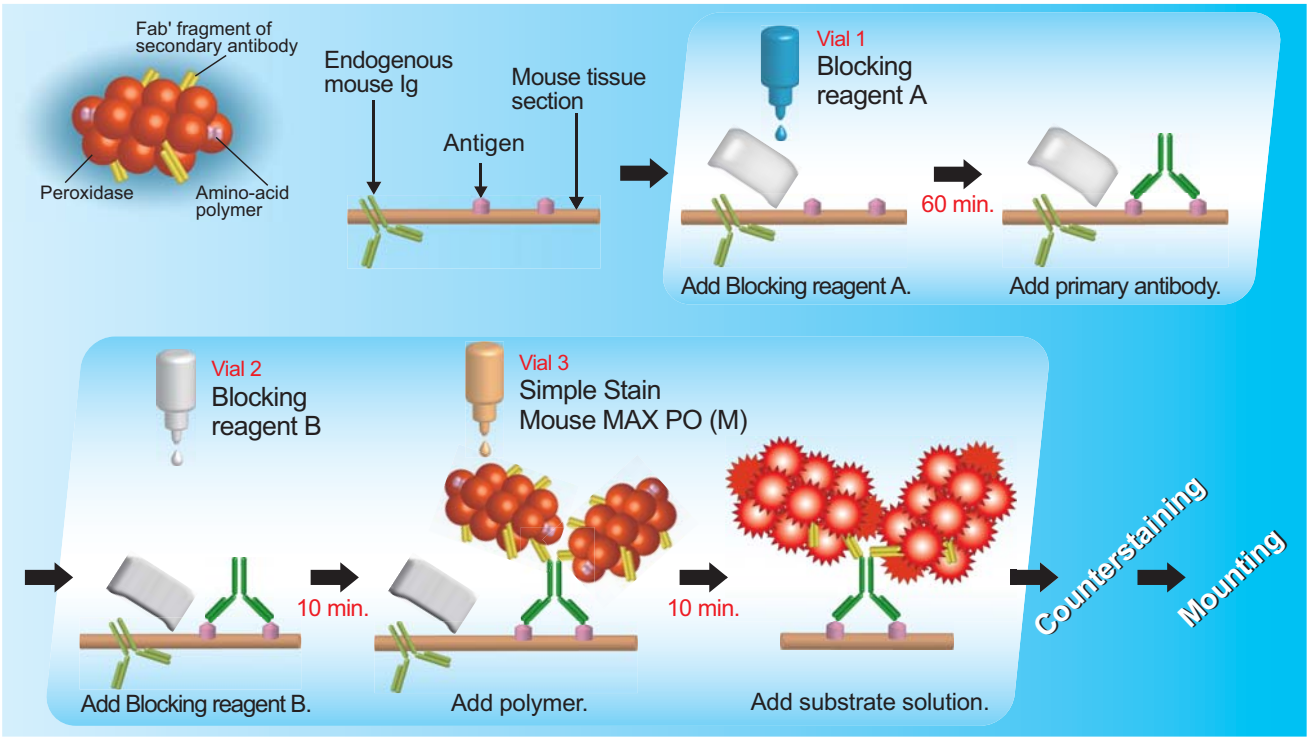
N-Histofine® MOUSESTAIN KIT *

This product adopted the UIP (Universal Immuno-enzyme Polymer) method, Nichirei Bio's patented technology.

Feature

N-Histofine® MOUSESTAIN KIT* is the mouse on mouse system. It is designed specifically to allow immunohistochemical staining with a mouse primary antibody on formalin-fixed paraffin-embedded mouse tissue sections. This kit consists of Blocking reagent A, Blocking reagent B, and Simple Stain Mouse Max PO (M) which is the labeled polymer prepared by combining amino acid polymer with multiple molecules of peroxidase and goat anti-mouse Ig which is reduced to Fab'. To eliminate background staining, this kit uses Blocking reagent A and Blocking reagent B.

Principle & Procedure

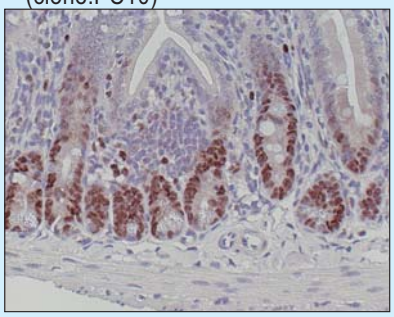


Advantages

1. No reaction to endogenous mouse immunoglobulin in the tissue.
2. Simplified staining steps (See page 12).
3. High sensitivity (See page 12).
4. Unaffected by endogenous biotin in the tissue (See page 12).
5. Ready to use.

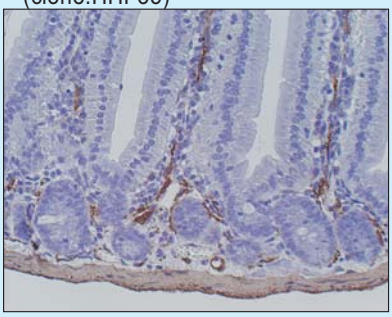
Staining Results

■Mouse anti-PCNA antibody (clone:PC10)



Mouse colon stained with N-Histofine® MOUSESTAIN KIT and DAB chromogen. Note nuclear staining of Epithelium cells.

■Mouse anti-Muscle Actin antibody (clone:HHF35)



Mouse colon stained with N-Histofine® MOUSESTAIN KIT and DAB chromogen. Note intense staining of smooth muscle and muscularis propria in lamina propria mucosae.

Product

Detection system.

Liquid. Ready to use.

Product Name	For (slides)	Volume	Code	For use with
N-Histofine® MOUSESTAIN KIT*	50	6ml x 3	414321F	Mouse primary antibody
	500	17ml x 9	414322F	

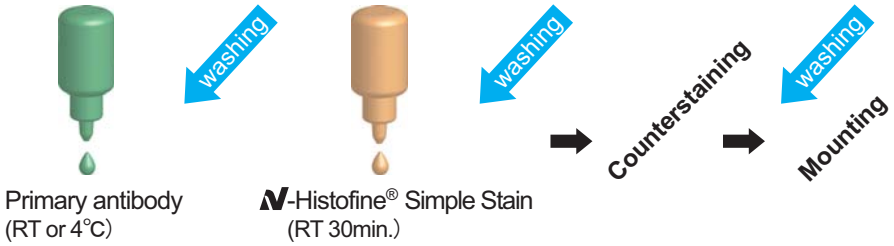
*: MOUSESTAIN KIT is not available in the United States.

Contents

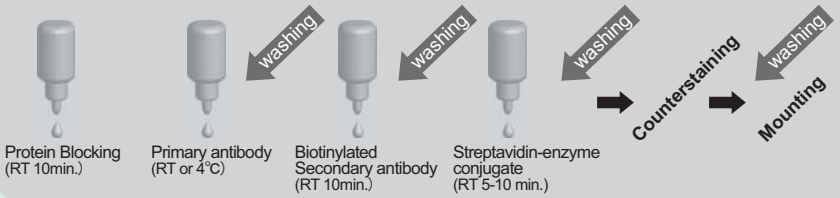
Advantages of N-Histofine® Simple Stain

Simplified staining steps

N-Histofine® Simple Stain

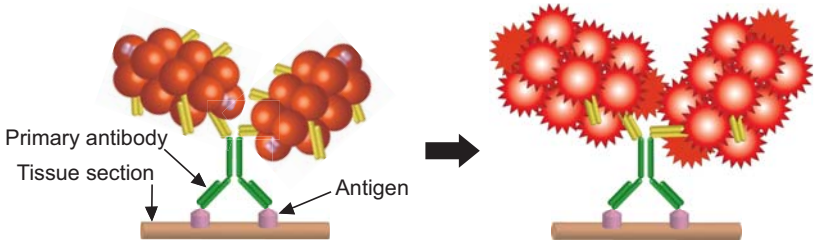


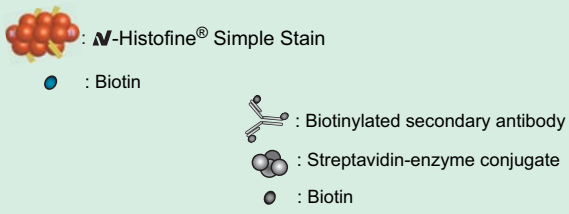
Conventional Streptavidin-biotin System



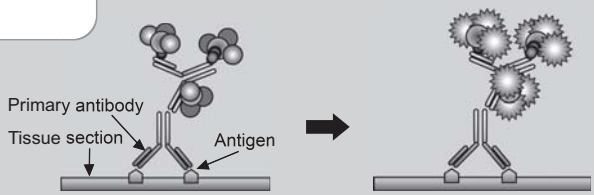
High sensitivity

N-Histofine® Simple Stain



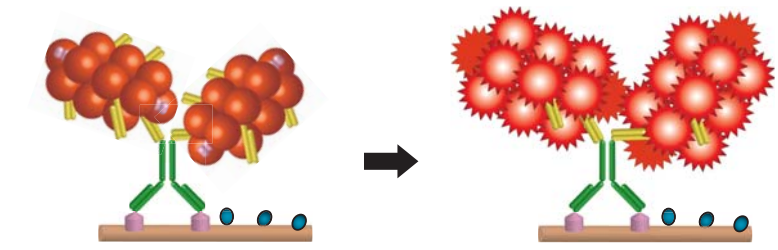


Conventional Streptavidin-biotin System

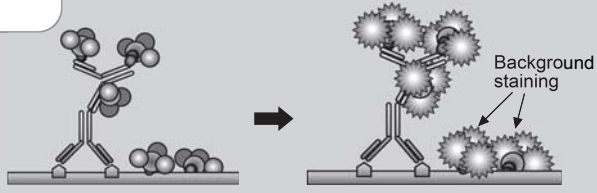


Unaffected by endogenous biotin in the tissue

N-Histofine® Simple Stain



Conventional Streptavidin-biotin System



Technical Report 1

Application of N-Histofine® MOUSESTAIN, Mouse MAX and Rat MAX for mouse and rat frozen tissue sections

N-Histofine® MOUSESTAIN, Mouse MAX and Rat MAX for mouse and rat, paraffin embedded tissue sections, are applicable for IHC staining with frozen tissue sections as well by following procedures.

Staining of frozen tissue sections with MOUSESTAIN KIT

MOUSESTAIN KIT is available for fixed frozen tissues without any change or addition on its procedure. (Principle & Procedure on page 11.)

1. Frozen tissue sections

Fixed frozen tissues* are only applicable.

*There are two different types of frozen tissues, Fresh frozen tissue and Fixed frozen tissue, used for IHC staining.
The fresh frozen tissue should be frozen immediately after the tissue obtained.
The fixed frozen tissue should firstly be fixed and then frozen.

2. Fixative solution

Apply fixative solution appropriate for primary antibody.

3. Concentration and reaction time of reagents

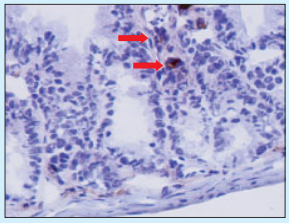
Apply equivalent concentration and reaction time of the respective reagents to these for paraffin embedded tissue sections.

In some preparation of frozen tissue sections, or regarding mouse lineages, tissues or fixing method, background staining may be observed in this regard.

Detection system : MOUSESTAIN KIT
Tissue sections : mouse normal colon
Fixative solution : 4% of PFA (at 4 °C for overnight)
Primary antibody : PBS (instead of the primary antibody)
Chromogen : DAB

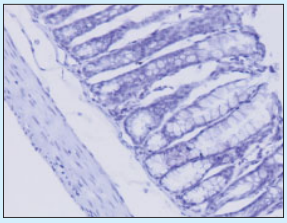
Staining Images

Fresh frozen tissue
4% PFA (4°C, 10 min.)



Background staining in plasma cells is observed.

Fixed frozen tissue
4% PFA (4°C, overnight)



Background staining in plasma cells is not observed.

Staining of frozen tissue sections with N-Histofine® Mouse MAX and Rat MAX for mouse and rat tissue sections

Following Step A firstly and Step B-A secondly are recommended before the reaction with substrate solution to eliminate background staining observed by the staining procedure. (Principle & Procedure on pages 9 and 10.)

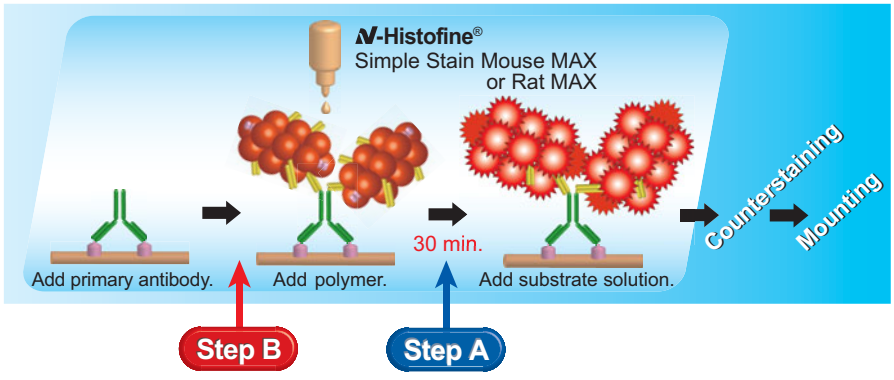
1. Frozen tissue sections

Both fixed frozen tissues and fresh frozen tissues are applicable.

2. Fixative solution

Apply fixative solution appropriate for primary antibody.

Staining procedure



Firstly **Step A** for background staining observed by this staining procedure

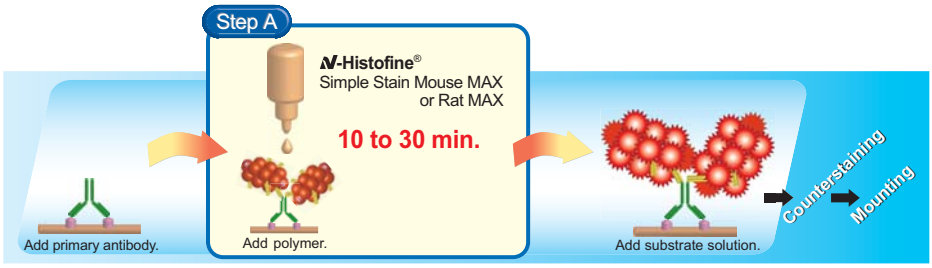
Secondly **Step B** **Step A** for background staining still observed by Step A

1. Step A Adjust reaction time of polymer.

30 min. of reaction time of polymer is designed for paraffin embedded sections.

For frozen tissue sections, apply adequate reaction time* reducing the duration between 10 to 30 min. when some background staining is observed by 30 min. reaction.

*The reaction time depends on mouse or rat lineages, tissues or fixing methods.

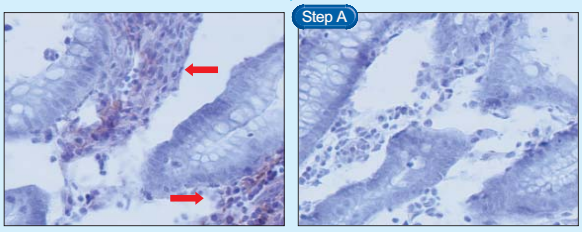


Staining Images

Background eliminated case by Step A

Detection system : Simple Stain Rat MAX PO (MULTI)
Tissue sections : rat normal colon
Fixative solution : 4% of PFA (at 4 °C for overnight)
Primary antibody : PBS (instead of the primary antibody)
Chromogen : DAB

Reaction time of polymer: 30 min. → Reaction time of polymer: 10 min.



Background staining in plasma cells is observed.

Background staining in plasma cells is not observed.

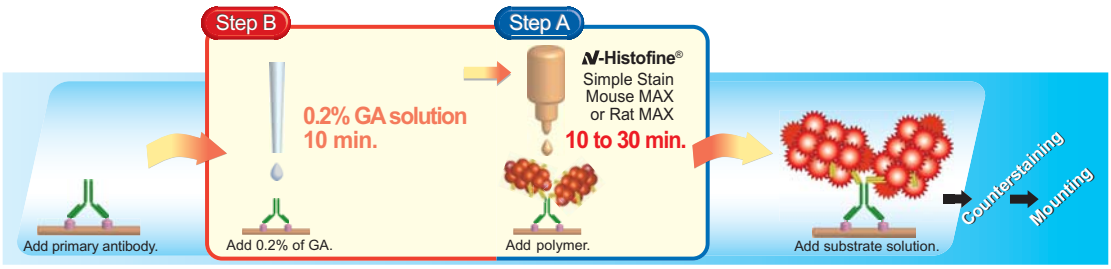
2. Step B Step A Add 0.2% of glutaraldehyde (GA) solution before the Step A.

Blocking with 0.2% of GA solution for 10 min.* may reduce background staining which is still observed by Step A.

Identify the absense of inhibition on the reaction of applied primary antibody prior to use of the GA solution.

*The effect of the blocking depends on mouse or rat lineages, tissues or fixing methods.

Dilution with SIGMA G7651 Dilute 250 times of SIGMA G7651, 50% concentration of GA, with PBS by 0.2% solution. Others should be equivalent to above dilution.

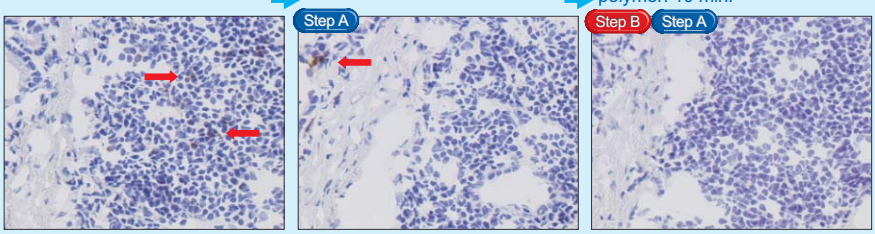


Staining Images

Background eliminated case by Step B-A

Detection system : Simple Stain Mouse MAX PO (R)
Tissue sections : mouse normal colon
Fixative solution : 4% of PFA (at 4 °C for 10 min.)
Primary antibody : PBS (instead of the primary antibody)
Chromogen : DAB

Reaction time of polymer: 30 min. → Reaction time of polymer: 10 min. → Reaction time of 0.2% GA: 10 min. polymer: 10 min.



Background staining in plasma cells is observed.

Background staining in plasma cells is reduced but slightly observed.

Background staining in plasma cells is not observed.

IHC Triple-staining Method

The IHC triple-staining method with N-Histofine® Simple Stain MAX PO (M), N-Histofine® Simple Stain AP (M) and three different murine primary antibodies

I. OBJECTIVE

Detection of three different antigens at different locations within the same tissue section

II. SPECIMENS

20% buffered formalin-fixed and paraffin-embedded tissue section

III. TECHNICAL ADVICE (Staining orders)

1st Detection of Antigen:
Detection of small amount of antigen with BCIP/NBT (Blue)

2nd Detection of Antigen:
Detection of cytoplasmic antigen or large amount of antigen with New Fuchsin (Red)

3rd Detection of Antigen:
Detection of nuclear antigen or moderate to large amount of antigen with DAB (Brown)

IV. STAINING PROCEDURES

1. Deparaffinization and Rehydration
   - 1-1. Immerse the slide in xylene at RT 3 times for 3 min each.
   - 1-2. Immerse the slide in 100% ethanol at RT 2 times for 3 min each.
   - 1-3. Immerse the slide in 95% ethanol at RT 2 times for 3 min each.
   - 1-4. Rinse the slide in PBS at RT for 5 min.

1st Detection of Antigen

2. Antigen Retrieval of the 1st primary antibody
   - 2-1. Refer to the package insert of the 1st primary antibodies and conduct proper antigen retrieval depend on the 1st primary antibody with specific buffer, specified temperature and incubation time, if necessary.
   - 2-2. Allow the slide to cool down at RT for 20 - 60 min. The slide should be cooled down slowly. Rinse the slide in PBS at RT 3 times for 5 min each.

3. Protein Blocking
   - Apply 10% Goat normal serum at RT for 10 min.

4. Add 1st Primary Antibody
   - 4-1. Apply 1st primary antibody at 37 ℃ for 1 hour.
   - 4-2. Rinse the slide in PBS at RT 3 times for 5 min each.

5. Add N-Histofine® Simple Stain AP (M)
   - 5-1. Apply N-Histofine® Simple Stain AP (M) at RT for 30 min.
   - 5-2. Rinse the slide in PBS at RT 3 times for 5 min each.
   - 5-3. Rinse the slide in TBS at RT for 5 min.

6. Add BCIP/NBT substrate
   - 6-1. Apply BCIP/NBT substrate solution. Adjust the incubation time by microscopic observation.
   - 6-2. Wash the slide with distilled water at RT for 5 min.

2nd Detection of Antigen

7. Antigen Retrieval of the 2nd primary antibody
   - 7-1. Conduct Method-A or Method-B depend on the 2nd primary antibody

   Method-A for the 2nd primary antibody NO Antigen Retrieval required
   1) Fill the heat-resistant plastic staining jar with 10 mM Sodium citrate buffer at pH 6.0 and heat to 95 ℃.
   2) Immerse the slide in the jar at 95 ℃ for 10 min*.

   *Inactivation treatment for the 1st primary antibody and the enzyme conjugated polymer of the 1st Detection of Antigen

   Method-B for the 2nd primary antibody Antigen Retrieval required
   1) Fill the heat-resistant plastic staining jar with the specific buffer instructed by the package insert of the 2nd primary antibody and heat to 95 ℃.
   2) Immerse the slide in the jar at 95 ℃ for 40 min**.
   3) Allow the slide to cool down at RT for 20-60 min. The slide should be cooled down slowly.

   **Activation treatment for the 2nd primary antibody of the 2nd Detection of Antigen, which is able to be combined with the treatment of above Method A

   - 7-2. Rinse the slide in PBS at RT 3 times for 5 min each.

8. Protein Blocking
   - Apply 10% Goat normal serum at RT for 10 min.

9. Add 2nd Primary Antibody
   - 9-1. Apply 2nd primary antibody at 37°C for 1 hour.
   - 9-2. Rinse the slide in PBS at RT 3 times for 5 min each.

10. Add N-Histofine® Simple Stain AP (M)
   - 10-1. Apply N-Histofine® Simple Stain AP (M) at RT for 30 min.
   - 10-2. Rinse the slide in PBS at RT 3 times for 5 min each.
   - 10-3. Rinse the slide in TBS at RT for 5 min.

11. Add New Fuchsin substrate
   - 11-1. Apply New Fuchsin substrate solution. Adjust the reaction time by microscopic observation.
   - 11-2. Wash the slide with distilled water at RT for 5 min.

3rd Detection of Antigen

12. Antigen Retrieval of the 3rd primary antibody
   - 12-1. Conduct method-A or method-B depend on the 3rd primary antibody

   Method-A for the 3rd primary antibody NO Antigen Retrieval required
   1) Fill the heat-resistant plastic staining jar with 10 mM Sodium citrate buffer at pH 6.0 and heat to 95 ℃.
   2) Immerse the slide in the jar at 95 ℃ for 10 min*.

   *Inactivation treatment for the 2nd primary antibody and the enzyme conjugated polymer of the 2nd Detection of Antigen

   Method-B for the 3rd primary antibody Antigen Retrieval required
   1) Fill the heat-resistant plastic staining jar with the specific buffer instructed by the package insert of the 3rd primary antibody and heat to 95 ℃.
   2) Immerse the slide in the jar at 95 ℃ for 40 min**.
   3) Allow the slide to cool down at RT for 20-60 min. The slide should be cooled down slowly.

   **Activation treatment for the 3rd primary antibody of the 3rd Detection of Antigen, which is able to be combined with the treatment of above Method A

   - 12-2. Rinse the slide in PBS at RT 3 times for 5 min each.

13. Quenching of endogenous peroxidase
13-1. Immerse the slide in 3% H_2O_2 solution in absolute methanol at RT for 10 min.
13-2. Rinse the slide in PBS at RT 3 times for 5 min each.
14. Protein Blocking
Apply 10% Goat normal serum at RT for 10 min.
15. Add 3rd Primary Antibody
15-1. Apply 3rd primary antibody at 37°C for 1 hour.
15-2. Rinse the slide in PBS at RT 3 times for 5 min each.
16. Add N-Histofine® Simple Stain MAX PO (M)
16-1. Apply N-Histofine® Simple Stain MAX PO (M) at RT for 30 min.
16-2. Rinse the slide in PBS at RT 3 times for 5 min each.
17. Add DAB substrate
17-1. Apply DAB substrate solution. Adjust the reaction time by microscopic observation.
17-2. Wash the slide with distilled water at RT for 5 min.

18. Mounting
During the slide are wet by water, put one drop of a water-soluble mounting media and fix with the cover slip.

V. STAINING RESULTS

Case 1: Human Reactive Lymph Node

1. OBJECTIVE
   Observe three types of stained cells in a tissue section.

2. SPECIMENS
   Human Reactive Lymph Node

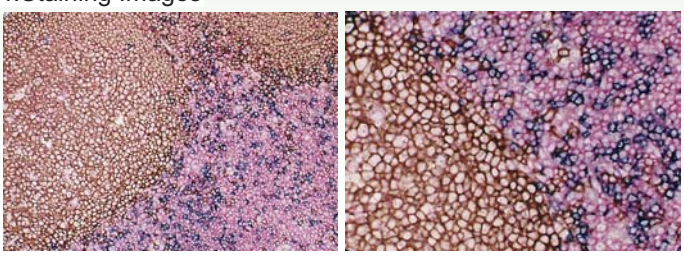
3. Used primary antibodies, antigen retrievals, detections and chromogens

1st Detection of Antigen:
- Primary Antibody: CD8
- Antigen Retrieval: 1mM buffered EDTA at pH8.0, 95 ℃ for 40 min.
- Detection system: N-Histofine® Simple Stain AP (M)
- Chromogen: BCIP/NBT (Blue)

2nd Detection of Antigen:
- Primary Antibody: CD4
- Antigen Retrieval: 1mM buffered EDTA at pH8.0, 95 ℃ for 40 min.
- Detection system: N-Histofine® Simple Stain AP (M)
- Chromogen: New Fuchsin (Red)

3rd Detection of Antigen:
- Primary Antibody: CD20cy
- Antigen Retrieval: 10mM Sodium citrate buffer at pH 6.0, 95 ℃ for 40 min.
- Detection system: N-Histofin® Simple Stain MAX PO (M)
- Chromogen: DAB (Brown)

4.Staining Images



Case 2: Cervical Squamous Cell Carcinoma

1. OBJECTIVE
   Observe three types of stained cells in a tissue section.

2. SPECIMENS
   Cervical Squamous Cell Carcinoma

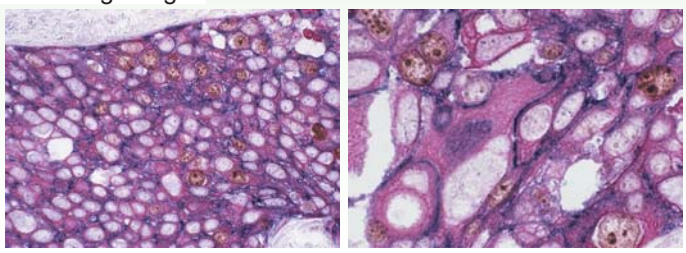
3. Used primary antibodies, antigen retrievals, detections and chromogens

1st Detection of Antigen:
- Primary Antibody: Beta-catenin
- Antigen Retrieval: 1mM buffered EDTA at pH8.0, 95 ℃ for 40 min.
- Detection system: N-Histofine® Simple Stain AP (M)
- Chromogen: BCIP/NBT (Blue)

2nd Detection of Antigen:
- Primary Antibody: Cytokeratin(AE1/AE3)
- Antigen Retrieval: 10mM Sodium citrate buffer at pH 6.0, 95 ℃ for 40 min.
- Detection system: N-Histofine® Simple Stain AP (M)
- Chromogen: New Fuchsin (Red)

3rd Detection of Antigen:
- Primary Antibody: Ki-67 antigen
- Antigen Retrieval: 10mM Sodium citrate buffer at pH 6.0, 95 ℃ for 40 min.
- Detection system: N-Histofin® Simple Stain MAX PO (M)
- Chromogen: DAB (Brown)

4.Staining Images



VI. ADVICE FOR STAINING

VI-1. Staining Schedule

Two-day separated completion of all the steps of the IHC triple-staining is available under the following conditions.

1st day
The reaction condition of 1st primary antibody should be at 4 ℃ for overnight.

2nd day
The reaction condition of both 2nd and 3rd primary antibodies should be at 37 ℃ for 1 hour.

VI-2. Chromogens Preparations

1. BCIP/NBT substrate solution

1-1. Reagents preparation

Substrate buffer (store at 2-8 °C):
100 mM Tris-HCl Buffer (100 mM sodium chloride, 50mM $MgCl_2$), pH 9.5
Adjust pH with HCl.

NBT stock solution (store at –20 °C):
Dissolve 75 mg of NBT (Nitro Blue Tetrazolium, SIGMA) in 1 ml of 70% N,N-dimethylformamid.

BCIP stock solution (store at –20 °C):
Dissolve 50 mg of BCIP (5-Bromo-4-Chloro-3-Indolyl Phosphate-p-Toluidine salt, SIGMA) in 1 ml of N,N-dimethylformamid.

1-2. Substrate solution preparation

Add 5 µl of BCIP stock solution and 6.5 µl of NBT stock solution to 1.5 ml of Substrate buffer and mix well.
Use the solution within 30 min after preparation.

2. New Fuchsin substrate solution

2-1. Reagents preparation

Naphthol AS-BI phosphate solution (Use within 30 min after preparation):
Dissolve 10 mg of naphthol AS-BI phosphoric acid (SIGMA) in 100 µl of N,N-dimethylformamid.

New Fuchsin solution (store at 2-8 °C):
Dissolve 4.0 g of New Fuchsin powder (MERCK) in 100 ml of 2N HCl and filter the solution.

4 % Sodium Nitrite solution (Use within 30 min after preparation):
Dissolve 40 mg of Sodium Nitrite in 1ml of distilled water

0.2 M Tris-HCl buffer (store at room temperature):
200mM Tris-HCl buffer, pH 8.2-8.3
Adjust pH with HCl.

2-2. Substrate solution preparation

Mix 100 µl of New Fuchsin solution and 100µl of 4 % Sodium Nitrite solution and incubate for 1 min.
Add 40ml of 0.2N Tris-HCl buffer to the mixture.
Add 100 µl of Naphthol AS-BI phosphate solution to the mixture while stirring constantly.
Use the solution immediately after Filtration.

3. DAB substrate solution

Dissolve and mix following reagents and stir the solution.
Use the solution within 30 min after preparation.

10 mg of 3,3'-Diaminobenzidine, tetrahydrochloride
50 ml of 0.05 M Tris-HCl buffer, 15mM NaN_3 pH 7.6
50 µl of 5 % H_2O_2 in distilled water
34 mg of Imidazole

Contents

Technical Report 3

Advantages of Immunohistochemical polymer detection systems designed for mouse & rat tissue sections

Introduction

When the immunohistochemical detection systems for human tissue sections are used for staining on mouse and rat tissue sections, background staining may be caused due to such reactivity with endogenous immunoglobulins of mouse and rat in the tissue sections. So that the immunohistochemical detection systems designed for staining on mouse and rat tissue sections were developed. Background staining is compared among those detection systems for mouse, rat and human tissue sections on mouse/rat tissue sections.

Materials & Methods

Materials

- Formalin-fixed paraffin-embedded mouse tissue sections
- Formalin-fixed paraffin-embedded rat tissue sections
- N-Histofine® Simple Stain Mouse MAX PO (Rat) :
  a polymer designed for staining with a rat primary antibody on mouse tissue sections
- N-Histofine® Simple Stain Rat MAX PO (M) :
  a polymer designed for staining with a mouse primary antibody on rat tissue sections
- N-Histofine® Simple Stain MAX PO (M) :
  a polymer designed for staining with a mouse primary antibody on human tissue sections

Methods

To compare background staining between polymers, immunohistochemical staining on mouse tissue sections was performed with N-Histofine® Simple Stain Mouse MAX PO (Rat) and N-Histofine® Simple Stain MAX PO (M). (Fig.1)

To compare background staining between polymers, immunohistochemical staining on rat tissue sections was performed with N-Histofine® Simple Stain Rat MAX PO (M) and N-Histofine® Simple Stain MAX PO (M). (Fig.2)

PBS was used instead of a primary antibody in order to identify the background staining caused by polymers. DAB solution was used for brown color development.

Steps of immunohistochemical staining

Quenching of endogenous peroxidase
↓
Incubation with PBS for 30 min.
↓
Incubation with polymer for 30 min.
↓
Incubation with DAB solution for 10 min.
↓
Counterstaining
↓
Mounting

Results

Fig.1 Mouse tissue sections

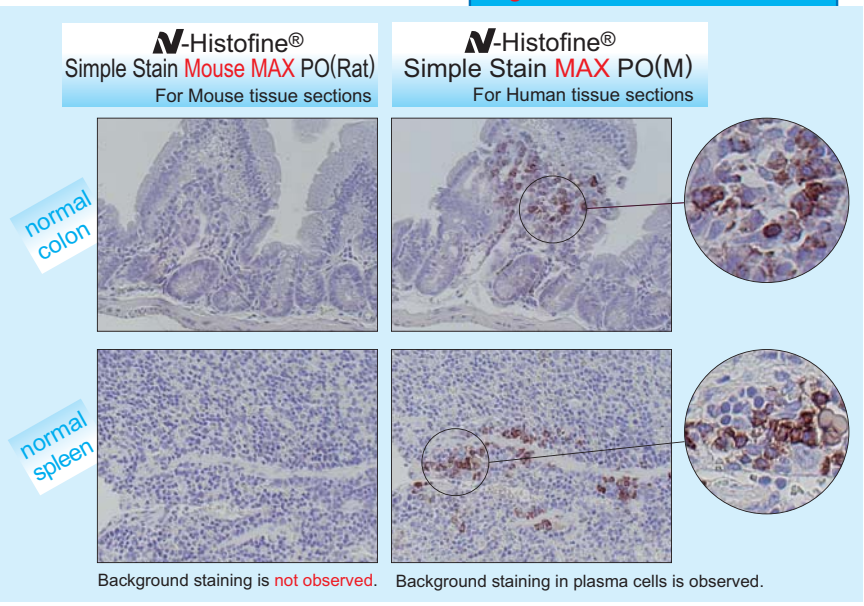
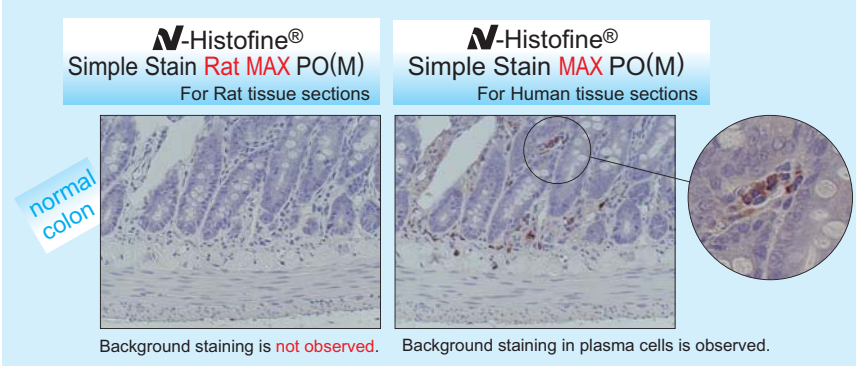


Fig.2 Rat tissue sections



Trouble Shooting

<table>
<tr><th>Problem</th><th>Possible cause</th><th>Solution</th></tr>
<tr><td rowspan="4">1. 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>・Never allow the tissue to dry out.</td></tr>
<tr><td>2) The embedding agent is not suitable, or paraffin is not thoroughly removed from paraffin-embedded sections.</td><td>・Select a suitable embedding agent or remove paraffin thoroughly from sections embedded.
・Change xylene or ethanol as the case may be.</td></tr>
<tr><td>3) Any trace amount of sodium azide present in the buffer inactivates the peroxidase, making the staining impossible.</td><td>・Use sodium azide free buffer solution.
・Change buffer solution.</td></tr>
<tr><td>4) Inadequate incubation of the enzyme and antibody.</td><td>・Change stale chromogen/substrate reagent.
・Blot off excess solution thoroughly at each stage.
・Provide sufficient time for reaction with antibody. In particular, primary antibody should be incubated for the time period specified in the package insert.</td></tr>
<tr><td rowspan="2">2. 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>・Some antigens are sensitive to fixation or embedding. So use less potent fixative and decrease the fixing time.
・The pretreatment is required for some tissues, in order to reveal the antigen, such as Antigen Recovery, Heat-Induced Epitope Retrieval or trypsin treatment.</td></tr>
<tr><td>2) Antigen is decomposed by autolysis.</td><td>・Use tissues obtained by biopsy or surgery, whenever possible.</td></tr>
<tr><td rowspan="12">3. The backgrounds are intensively stained in all the slides.</td><td>Peroxidase staining
1) Endogenous enzyme activity was not completely blocked.</td><td>・Ensure the treatment with 3% of hydrogen peroxide added methanol to inactivate endogenous peroxidase activity.</td></tr>
<tr><td>2) Non-specific staining is found.</td><td>・Before adding primary antibody, treat with 10% normal goat or rabbit serum as follows.
<table>
<tr><th>Product name</th><th>Serum</th></tr>
<tr><td>Simple Stain MAX PO (M)</td><td>goat</td></tr>
<tr><td>Simple Stain MAX PO (R)</td><td>goat</td></tr>
<tr><td>Simple Stain MAX PO (MULTI)</td><td>goat</td></tr>
<tr><td>Simple Stain MAX PO (G)</td><td>rabbit</td></tr>
<tr><td>Simple Stain Mouse MAX PO (R)</td><td>goat</td></tr>
<tr><td>Simple Stain Mouse MAX PO (G)</td><td>rabbit</td></tr>
<tr><td>Simple Stain Mouse MAX PO (Rat)</td><td>goat</td></tr>
<tr><td>Simple Stain Rat MAX PO (M)</td><td>goat</td></tr>
<tr><td>Simple Stain Rat MAX PO (R)</td><td>goat</td></tr>
<tr><td>Simple Stain Rat MAX PO (G)</td><td>rabbit</td></tr>
<tr><td>Simple Stain Rat MAX PO (MULTI)</td><td>goat</td></tr>
</table></td></tr>
<tr><td>Alkaline phosphatase staining
1) Endogenous enzyme activity was not completely blocked.</td><td>・Add Levamisole to chromogen/substrate solution. To reduce endogenous enzyme activity, chromogen/substrate solution containing 1mM Levamisole should be used.</td></tr>
<tr><td>2) Non-specific staining is found.</td><td>・Before adding primary antibody, treat with 10% normal goat serum.
<table>
<tr><th>Product name</th><th>Serum</th></tr>
<tr><td>Simple Stain AP (M)</td><td>goat</td></tr>
<tr><td>Simple Stain AP (R)</td><td>goat</td></tr>
<tr><td>Simple Stain AP (MULTI)</td><td>goat</td></tr>
</table></td></tr>
<tr><td>3) Autolysis results in excessive antigens isolated in histological solutions.</td><td>・Obtain fresh tissues whenever available.</td></tr>
<tr><td>4) Insufficient removal of paraffin.</td><td>・Change xylene or ethanol as the case may be.</td></tr>
<tr><td>5) Insufficient washing of antibody.</td><td>・Ensure thorough washing of antibody.</td></tr>
<tr><td>6) A high room temperature accelerates enzyme reactions.</td><td>・Keep room temperature at 15 to 25°C.
・Shorten reaction time of enzyme.</td></tr>
<tr><td>7) Drying-out of specimens during staining after addition of the reagents.</td><td>・Never allow the tissue to dry out.</td></tr>
<tr><td>4. 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>・Mount tissue sections on slides coated with an adhesive such as 0.02% poly-L-lysine or silane.</td></tr>
</table>

Contents

References

Human Tissue Sections

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(2) Kitada, M., et al：Translocation of Glomerular p47phox and p67phox by Protein Kinase C-β Activation Is Required for Oxidative Stress in Diabetic Nephropathy. Diabetes 52：2603–2614, 2003.
(3) Matsuyoshi, H., et al：Enhanced Priming of Antigen-Specific CTLs In Vivo by Embryonic Stem Cell-Derived Dendritic Cells Expressing Chemokine Along with Antigenic Protein: Application to Antitumor Vaccination. The Journal of Immunology 172：776-786, 2004.
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Product List

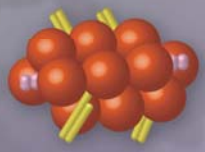
Product Name	For (slides)	Volume	Code	For use with	Mark in EU
For Human Tissue Sections					
N-Histofine® Simple Stain MAX PO (MULTI)	170	17ml x 1	414151F	Mouse and rabbit primary antibodies	CE
	500	17ml x 3	414152F		
	1500	17ml x 9	414154F		
N-Histofine® Simple Stain MAX PO (M)	170	17ml x 1	414131F	Mouse primary antibody	
	500	17ml x 3	414132F		
	1500	17ml x 9	414134F		
N-Histofine® Simple Stain MAX PO (R)	170	17ml x 1	414141F	Rabbit primary antibody	
	500	17ml x 3	414142F		
	1500	17ml x 9	414144F		
N-Histofine® Simple Stain MAX PO (G)	170	17ml x 1	414161F	Goat primary antibody	
	500	17ml x 3	414162F		
N-Histofine® Simple Stain AP (MULTI)	170	17ml x 1	414261F	Mouse and rabbit primary antibodies	
	500	17ml x 3	414262F		
N-Histofine® Simple Stain AP (M)	170	17ml x 1	414241F	Mouse primary antibody	
	500	17ml x 3	414242F		
N-Histofine® Simple Stain AP (R)	170	17ml x 1	414251F	Rabbit primary antibody	
	500	17ml x 3	414252F		
For Mouse Tissue Sections					
N-Histofine® Simple Stain Mouse MAX PO (R)	170	17ml x 1	414341F	Rabbit primary antibody	
N-Histofine® Simple Stain Mouse MAX PO (G)	170	17ml x 1	414351F	Goat primary antibody	
N-Histofine® Simple Stain Mouse MAX PO (Rat)	170	17ml x 1	414311F	Rat primary antibodies	
N-Histofine® MOUSESTAIN KIT*	50	6ml x 3	414321F	Mouse primary antibody	
	500	17ml x 9	414322F		
Blocking Reagent A Blocking Reagent B Simple Stain Mouse Max PO (M)	* MOUSESTAIN KIT is not available in the United States.				
For Rat Tissue Sections					
N-Histofine® Simple Stain Rat MAX PO (MULTI)	170	17ml x 1	414191F	Mouse and rabbit primary antibodies	
N-Histofine® Simple Stain Rat MAX PO (M)	170	17ml x 1	414171F	Mouse primary antibody	
N-Histofine® Simple Stain Rat MAX PO (R)	170	17ml x 1	414181F	Rabbit primary antibody	
N-Histofine® Simple Stain Rat MAX PO (G)	170	17ml x 1	414331F	Goat primary antibody	
Enzyme Substrate Systems					
N-Histofine® DAB-3S kit	500	3ml x 3	415192F	Peroxidase	CE
	1500	9ml x 3	415194F		
Reagent A : DAB solution concentrate Reagent B : Enhancer solution concentrate Reagent C : Hydrogen peroxide solution concentrate					
N-Histofine® Simple Stain AEC Solution	500	17ml x 3	415182F	Peroxidase	
	1500	17ml x 9	415184F		
N-Histofine® New Fuchsin Substrate kit	2000	* 6ml x 3 **12ml x 1	415161F	Alkaline phosphatase	
Reagent A : New Fuchsin solution concentrate* Reagent B : Activating reagent concentrate* Reagent C : Substrate buffer concentrate** Reagent D : Substrate solution concentrate*					

Liquid. Ready to use.

Product Name	Size	Volume	Code	For use with
For Human Tissue Sections				
N-Histofine® ALK Detection Kit	20 tests		417071F	
1 Blocking Reagent		4ml × 1		
2 Primary Antibody		2ml × 1		
3 Negative Control		2ml × 1		
4 Bridge Reagent		4ml × 1		
5 Peroxidase Labeled Empower Reagent		4ml × 1		
6 Chromogen Substrate		0.5ml × 1		
7 Substrate Buffer Solution		0.5ml × 1		
8 Chromogen Reagent		0.5ml × 1		
9 ALK Antigen Retrieval Solution A		150ml × 1		
10 ALK Antigen Retrieval Solution B		150ml × 1		
N-Histofine® ALK Control Slides	5 slides		417081F	

Storage

Store in a dark place at 2 to 8℃.



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