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 14.)

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

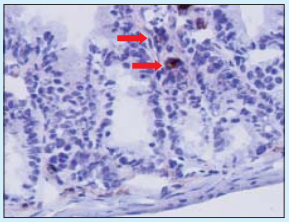
Apply equivalent concentration and reaction time of the respective reagents to these for paraffin embedded tissue sections.

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

Staining Images

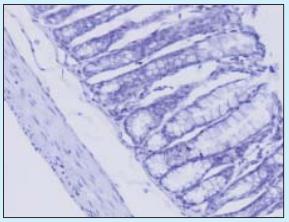
Detection system : MOUSESTAIN KIT
Tissue sections : mouse normal colon
Fixative solution : 4% of PFA (at 4 °C for overnight)
Primary antibody : PBS (in place of the primary antibody)
Chromogen : DAB

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 12 and 13.)

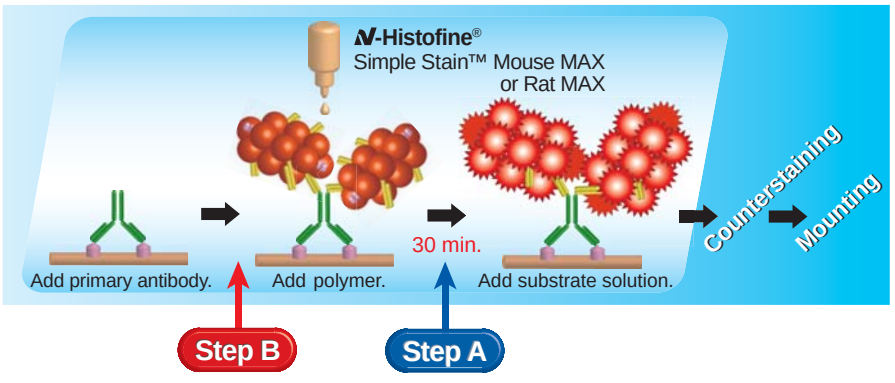
1. Frozen tissue sections

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

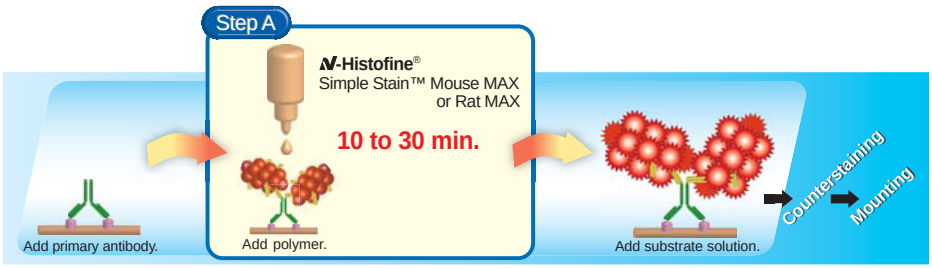
Secondly **Step B** **Step A** for background staining still observed by Step A

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.

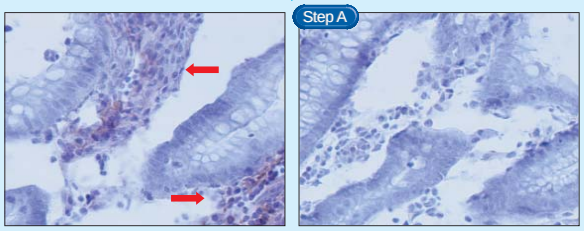


Staining Images

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 (in place of 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.

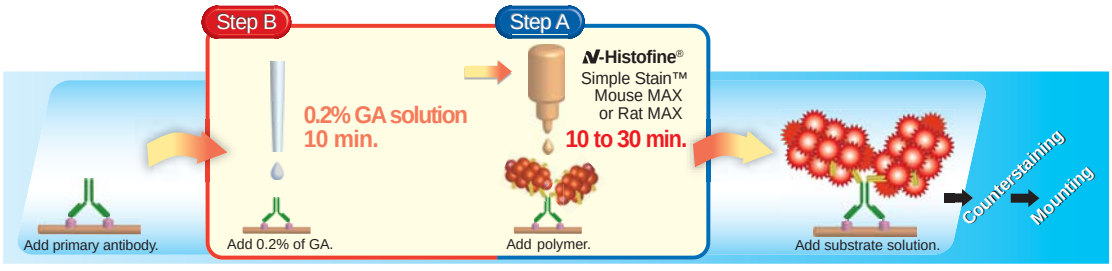
2. Step B Step A Add 0.2% of glutaraldehyde (GA) solution before the Step A.

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.

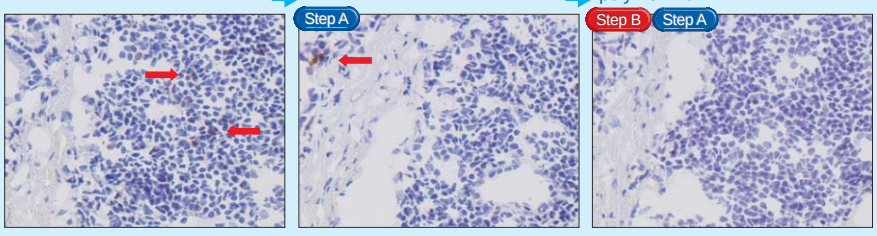


Staining Images

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 (in place of 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.

Contents