Cat. # MK200-205

For Research Use

TaKaRa

TaKaRa POD Conjugate For Mouse Tissue/For Tissue

Product Manual

v201608

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I. Description

TaKaRa POD Conjugate is a reagent designed for immunohistochemical staining of paraffin-embedded tissue sections. This product consists of an amino acid polymer combined with peroxidase and Fab' fragments of a secondary antibody. It is supplied at a concentration suitable for immediate use, in MOPS (3-(*N*-MorpH olino) propanesulfonic acid) buffer (pH 6.5) containing stabilizing protein and antibiotics. This product provides clear, highly sensitive results with minimal background staining, and is simpler and easier to use than the streptavidin-biotin method.
This product is available in two different formats, "For Mouse Tissue" (for mouse tissue sections) and "For Tissue" (for tissue sections from other mammalian sources). Three specific versions are available in each format, based on the source species of the primary antibody. "Anti Mouse," "Anti Rat" and "Anti Rabbit" versions are available "For Mouse Tissue," and "Anti Mouse," "Anti Rabbit" and "Anti Goat" versions are available "For Tissue." The TaKaRa POD Conjugate Set Anti Mouse, For Mouse Tissue (Cat. #MK200) is unique because it may be used with a mouse primary antibody on mouse tissue. It consists of Blocking Reagents A and B and TaKaRa POD Conjugate Anti Mouse, For Mouse Tissue. The "For Tissue" products can be used with tissue sections from various species, but it is important to determine in advance the appropriate conditions for each tissue and primary antibody.

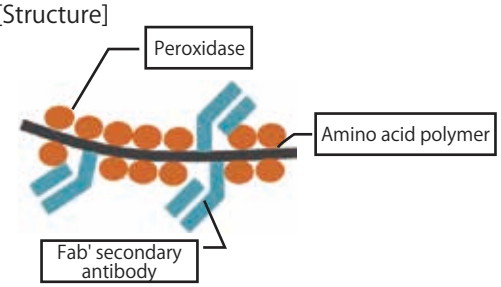
Features

- It does not react with endogenous immunoglobulins (Ig).
- It is not affected by endogenous biotin.
- There is no need for a blocking reaction or for adding an enzyme-conjugated reagent that reacts with the secondary antibody as required in the streptavidin-biotin (SAB) method.*

*:TaKaRa POD Conjugate Set Anti Mouse, For Mouse Tissue requires a blocking reaction against endogenous mouse Ig using the reagents in this product.

II. Principle

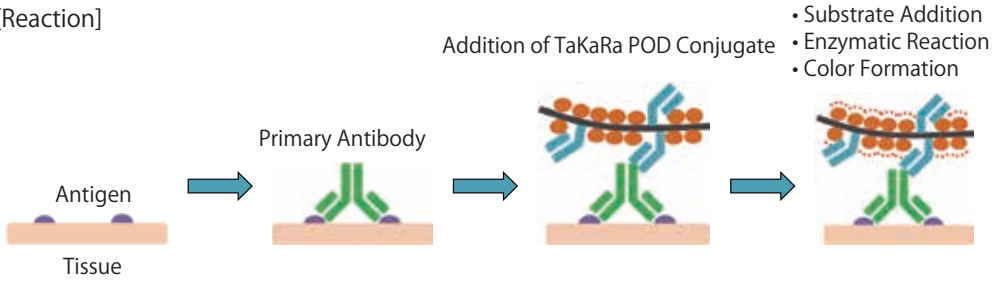
[Structure]



		Fab' secondary antibody (Source species)
TaKaRa POD Conjugate	Anti Mouse	Anti Mouse IgG (Goat)
	Anti Rat	Anti Rat IgG (Goat)
	Anti Rabbit	Anti Rabbit IgG (Goat)
	Anti Goat	Anti Goat IgG (Rabbit)

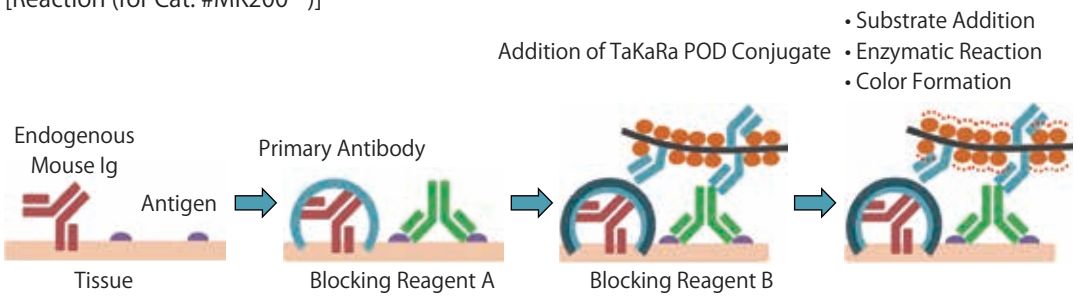
TaKaRa POD Conjugate consists of an amino acid polymer combined with peroxidase and Fab' fragments of a secondary antibody.

[Reaction]



After the primary antibody reacts with the antigen on a tissue section, the labeled polymers in TaKaRa POD Conjugate bind to the primary antibody-antigen to form a complex. The target antigen is then developed by adding a colorimetric substrate that reacts with the peroxidase in the TaKaRa POD Conjugate.

[Reaction (for Cat. #MK200*)]



TaKaRa POD Conjugate Set Anti Mouse, For Mouse Tissue (Cat. #MK200)* is designed for staining mouse tissues using a mouse primary antibody. Treatment with Blocking Reagents A and B prevents the primary antibody from reacting with endogenous immunoglobulins (Ig), and suppresses background staining. As a result, only the target antigen is stained.

* :Not available in all geographic locations. Check for availability in your area.

III. Components

Cat. #	Name and Components	Volume (for 60 reactions)	Target Tissues	Primary Antibody Source
MK200*	TaKaRa POD Conjugate Set Anti Mouse, For Mouse Tissue Components • Blocking Reagent A • Blocking Reagent B • TaKaRa POD Conjugate Anti Mouse, For Mouse Tissue	1 set 6 ml 6 ml 6 ml	Mouse	Mouse
MK201	TaKaRa POD Conjugate Anti Rat, For Mouse Tissue	6 ml		Rat
MK202	TaKaRa POD Conjugate Anti Rabbit, For Mouse Tissue	6 ml		Rabbit Guinea Pig
MK203	TaKaRa POD Conjugate Anti Goat, For Tissue	6 ml	Tissues other than mouse	Goat
MK204	TaKaRa POD Conjugate Anti Mouse, For Tissue	6 ml		Mouse
MK205	TaKaRa POD Conjugate Anti Rabbit, For Tissue	6 ml		Rabbit Guinea Pig

*: Not available in all geographic locations. Check for availability in your area.

Each of these products can be used as a labeled secondary antibody in an immunohistochemical staining of paraffin-embedded tissue sections. They are designed for specific staining of a target antigen in a short period of time. These products should be used at the supplied concentration, without dilution. The standard amount per use is 2 drops for a 1 cm x 1 cm tissue section. One drop from the reagent bottle corresponds to 40 - 45 μl.

[TaKaRa POD Conjugate Set Anti Mouse, For Mouse Tissue (Cat. #MK200)]

This product is designed for staining mouse tissues using a mouse primary polyclonal or monoclonal antibody. It provides specific staining of the target antigen by using blocking reagents (Blocking Reagent A, Blocking Reagent B) before and after the primary antibody reaction to inhibit a reaction with endogenous mouse immunoglobulin (Mouse Ig), and to suppress background staining.

NOTE: This product is not available in all geographic locations. Check for availability in your area.

● Components

- TaKaRa POD Conjugate Anti Mouse, For Mouse Tissue (in a dropper bottle): 6 ml
  [Goat Anti Mouse Ig-Fab - Peroxidase Conjugate,
  MOPS (3-(*N*-MorpH olino) propanesulfonic acid) buffer (pH 6.5),
  Bovine Serum Albumin, and preservatives]
- Blocking Reagent A (in a dropper bottle): 6 ml
  [protein solution containing 0.1% sodium azide]
- Blocking Reagent B (in a dropper bottle): 6 ml
  [reagent solution containing 0.1% sodium azide]

[TaKaRa POD Conjugate Anti Rat, For Mouse Tissue (Cat. #MK201)]

This product is used for staining mouse tissue with a rat primary antibody.

● Components

- TaKaRa POD Conjugate Anti Rat, For Mouse Tissue (in a dropper bottle): 6 ml
  [Goat Anti Rat Ig-Fab - Peroxidase Conjugate,
  MOPS (3-(*N*-morpH olino) propanesulfonic acid) buffer (pH 6.5),
  Bovine Serum Albumin, and preservatives]

[TaKaRa POD Conjugate Anti Rabbit, For Mouse Tissue (Cat. #MK202)]

This product is used for staining mouse tissue with a rabbit primary antibody.

● Components

- TaKaRa POD Conjugate Anti Rabbit, For Mouse Tissue (in a dropper bottle): 6 ml
  [Goat Anti Rabbit Ig-Fab - Peroxidase Conjugate,
  MOPS (3-(*N*-morpH olino) propanesulfonic acid) buffer (pH 6.5),
  Bovine Serum Albumin, and preservatives]

[TaKaRa POD Conjugate Anti Goat, For Tissue (Cat. #MK203)]

This product is used for staining various species of animal tissue other than mouse with a goat primary antibody.

● Components

- TaKaRa POD Conjugate Anti Goat, For Tissue (in a dropper bottle): 6 ml
  [Rabbit Anti Goat Ig-Fab - Peroxidase Conjugate,
  MOPS (3-(*N*-morpH olino) propanesulfonic acid) buffer (pH 6.5),
  Bovine Serum Albumin, and preservatives]

[TaKaRa POD Conjugate Anti Mouse, For Tissue (Cat. #MK204)]

This product is used for staining various species of animal tissue other than mouse with a mouse primary antibody.

● Components

- TaKaRa POD Conjugate Anti Mouse, For Tissue (in a dropper bottle): 6 ml
  [Goat Anti Mouse Ig-Fab - Peroxidase Conjugate,
  MOPS (3-(*N*-morpH olino) propanesulfonic acid) buffer (pH 6.5),
  Bovine Serum Albumin, and preservatives]

[TaKaRa POD Conjugate Anti Rabbit, For Tissue (Cat. #MK205)]

This product is used for staining various species of animal tissue other than mouse with a rabbit primary antibody.

● Components

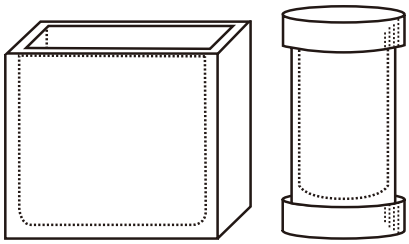
- TaKaRa POD Conjugate Anti Rabbit, For Tissue (in a dropper bottle): 6 ml
  [Goat Anti Rabbit Ig-Fab - Peroxidase Conjugate,
  MOPS (3-(*N*-morpH olino) propanesulfonic acid) buffer (pH 6.5),
  Bovine Serum Albumin, and preservatives]

IV. Materials Required but not Provided

Equipment

- Glass slide
- Container for staining＊1
- Dryer
- Container for washing
- Timer
- Slide stand
- Wet box
- Cover glass
- Tissue paper
- Microscope

＊1 : Container for staining (see examples below). Use containers that are transparent, solvent-resistant and made of glass or plastic, etc.



Reagents

- Xylene
- 95% Ethanol
- 100% Ethanol
- PBS (For washing)
- 3% Hydrogen peroxide with methanol＊2
- Primary antibody
- Negative control (normal serum of animal species from which the primary antibody is generated)
- Substrate solution (DAB or AEC solution)
  TaKaRa DAB Substrate (Cat. #MK210) etc.
- Counterstaining reagents (hematoxylin, methyl green, etc.)
- Mounting medium
- Glue for tissue sections (as necessary)
- Antigen activation liquid
- Distilled water

＊2 : 3% Hydrogen peroxide with methanol.
30% Hydrogen peroxide is diluted 10-fold with methanol (Prepare immediately before use).

V. Storage 4℃, in an aluminum pouch to protect reagents from light.

VI. Intended Use

This product consists of reagents for immunohistochemical staining of paraffin-embedded sections. It is possible to stain frozen sections, but this requires optimization of the staining method. (Refer to Section IX.1. Frozen Section Immunostaining)

VII. Protocol

1. Sample Preparation

Paraffin-embedded tissue sections

Slice each sample into 3 - 6 μm thick sections, and attach them to slides. When antigen retrieval is performed, use surface-treated slides (0.02% poly-L-lysine or silane, etc.) to prevent detachment of the section.

Sample slides

Prepare two sample slides for each sample.

- Sample slide
  Staining is performed using the target primary antibody.
- Sample slide for control reaction
  Staining is performed using a negative control (normal serum of the animal species from which the primary antibody is generated, etc.) instead of the primary antibody.

Control slides

Prepare control slides. The entire process, from staining to microscopic examination, should be performed with sample slides in parallel with reagent control slides.

- Positive control slide
  A tissue section slide that has been preliminarily validated to express the target antigen.
- Negative control slide
  A tissue section slide that has been preliminarily validated to not express the target antigen.

[Precaution]

- Antigens are sensitive to heat. When the tissue is mounted, do not heat the paraffin above 58℃.
- Steroids and other small molecules are very soluble in organic solvents. The fixative must be carefully selected to prevent antigen loss.

2. Preparation of Reagents

Every reagent in this product (Cat. #MK200 - MK205) is provided at a concentration suitable for immediate use and should not be diluted.

3. Procedure

Unless otherwise specified, the steps in the following procedure are performed at room temperature (15 - 25℃).

Immunohistochemical staining procedure using TaKaRa POD Conjugate

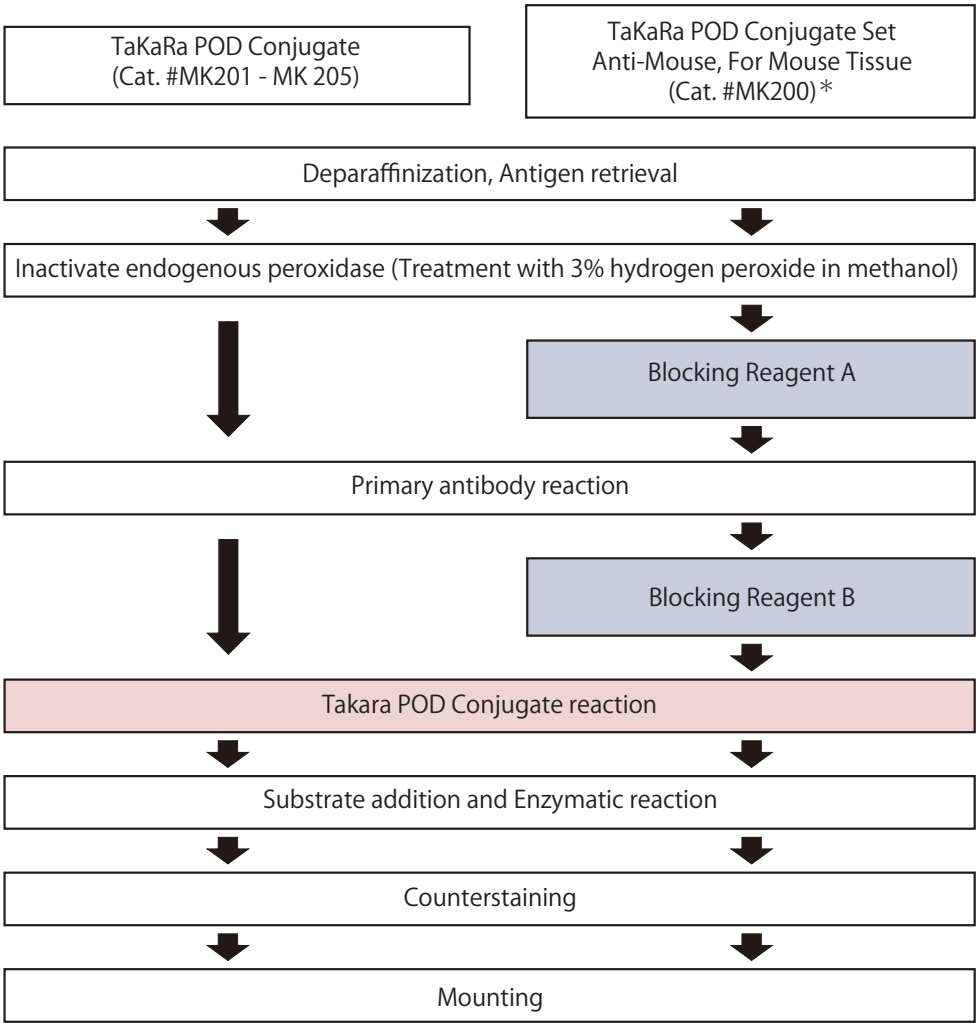


Figure 1. Workflow

*:Not available in all geographic locations. Check for availability in your area.

A. Deparaffinization

Table 1. Deparaffinization Procedure

1	Xylene 1 bath	3 min
2	Xylene 2 bath	3 min
3	Xylene 3 bath	3 min
4	100% (Absolute) Ethanol 1 bath	3 min
5	100% (Absolute) Ethanol 2 bath	3 min
6	95% Ethanol 1 bath	3 min
7	95% Ethanol 2 bath	3 min
8	PBS wash	3 x 3 min

- Prepare three xylene baths in separate staining containers.
- Prepare two 100% (absolute) ethanol baths in separate staining containers.
- Prepare two 95% ethanol baths in separate staining containers.
- Prepare PBS in a single staining container.
- Make sure to use enough of each reagent to cover the paraffin-containing regions of the tissue sections.

＊ Use organic solvents with appropriate ventilation, such as a fume hood.

(1) Xylene Treatment

1) Soak the slides in the Xylene 1 bath for 3 min.
2) Remove any extra solution and soak the slides in the Xylene 2 bath for 3 min.
3) Remove any extra solution, soak the slides in the Xylene 3 bath for 3 min, and follow with ethanol treatment.

(2) Ethanol Treatment

1) Soak the slides in the 100% (Absolute) Ethanol 1 bath for 3 min.
2) Remove any extra solution and soak the slides in the 100% (Absolute) Ethanol 2 bath for 3 min.
3) Remove any extra solution and soak the slides in the 95% Ethanol 1 bath for 3 min.
4) Remove any extra solution and soak the slides in the 95% Ethanol 2 bath for 3 min.

(3) Washing

1) Remove any extra solution and wash the slides by soaking in PBS for 3 min.
2) After changing PBS, wash the slides by soaking in fresh PBS for 3 min.
3) Repeat Step 2 one more time (for a total of 3 PBS washes).＊

＊:Step 3 may be omitted if Section VII.3.C(1): Inactivation of endogeous peroxidase is performed.

Notes:

- Before xylene treatment, the paraffin is easily melted by warming the slides to 40 - 50℃ on a slide warmer or block incubator, etc. for about 10 min.
- Change the xylene and ethanol in the baths when they become dirty or deteriorated. We recommend changing these solutions periodically.

B. Antigen Retrieval

Antigen retrieval and proteinase treatment should be performed as needed using conditions that are optimal for the primary antibody. For general antigen retrieval protocols, refer to Section VIII.2. Antigen retrieval.

C. Immunostaining

- Do not let the sections dry out during the staining process. Prevent drying by using a wet box or coverslips.
- Bring the reagents to room temperature before use.

(1) Inactivation of endogenous peroxidase (treatment with 3% hydrogen peroxide in methanol)

Prepare 3% hydrogen peroxide fresh just before use.

1) Remove any extra water around the sections.
2) Cover the sections completely with 3% hydrogen peroxide in methanol and incubate at room temperature (15 - 25℃) for 10 - 15 min.
3) After removing the extra solution, wash the sections by soaking in PBS for 3 min.
4) After changing PBS, wash the sections by soaking in fresh PBS for 3 min.
5) Repeat Step 4 one more time (for a total of 3 PBS washes).

(2) Treatment with Blocking Reagent A

This step is necessary only for TaKaRa POD Conjugate Set Anti Mouse, For Mouse Tissue (Cat. #MK200).＊1

1) Remove any extra water around the sections.
2) Cover the sections completely with Blocking Reagent A and incubate at room temperature for 60 min Protect them from drying using coverslips, etc.
3) Wash the sections by soaking in PBS for 3 min.
4) After changing PBS, wash the sections by soaking in fresh PBS for 3 min.
5) Repeat Step 4 one more time (for a total of 3 PBS washes).

(3) Primary antibody reaction

1) Remove any extra water around the sections.
2) Use an antibody concentration, reaction temperature, and reaction time that is optimal for your primary antibody.
   Completely cover your sample sections, as well as positive and negative control sections, with the same primary antibody.
   For a negative control reaction, use normal serum from the source species of the primary antibody in place of the primary antibody.
   Incubate at the room temperature for 1 - 2 hours or at 4℃ overnight.
3) Wash the sections by soaking in PBS for 3 min.
4) After changing the PBS, wash the sections by soaking in fresh PBS for 3 min.
5) Repeat Step 4 one more time (for a total of 3 PBS washes).

(4) Treatment with Blocking Reagent B

This procedure is necessary only for TaKaRa POD Conjugate Set Anti Mouse, For Mouse Tissue (Cat. #MK200).＊1

1) Remove any extra water around the sections.
2) Cover the sections completely with Blocking Reagent B and incubate at room temperature for 10 min.
3) Wash the sections by soaking in PBS for 3 min.
4) After changing PBS, wash the sections by soaking in fresh PBS for 3 min.
5) Repeat Step 4 one more time (for a total of 3 PBS washes)

＊1 : Not available in all geographic locations. Check for availability in your area.

(5) Reaction with TaKaRa POD Conjugate

Select the appropriate TaKaRa POD Conjugate based on the source species of the sample tissue and the source species of the primary antibody.

1) Remove any extra water around the sections.
2) Cover the sections completely with TaKaRa POD Conjugate (2 drops for a 1 cm x 1 cm tissue section) and incubate at room temperature for 30 min.＊2
3) Wash the sections by soaking in PBS for 3 min.
4) After changing PBS, wash the sections by soaking in fresh PBS for 3 min.
5) Repeat Step 4 one more time (for a total of 3 PBS washes).

＊2 : If the background is high, adjust the reaction time.

(6) Reaction with substrate solution

Select a suitable substrate (Refer to Section VIII.1. Selection of Substrate, Mounting Medium, and Counterstaining Reagents.)

1) Remove any extra water around the sections.
2) Cover the sections completely with substrate solution (DAB or AEC) and incubated at room temperature for 5 - 20 min.
3) Rinse with distilled water.

D. Counterstaining

Select a suitable counterstaining reagent (Refer to Section VIII.1. Selection of Substrate, Mounting Medium, and Counterstaining Reagents.)

(1) Soak slides in counterstaining reagent. Adjust the staining time and dilution of counterstaining reagent to achieve a reasonable level of staining.
(2) Wash with running water (tap water) for 10 min, then rinse away impurities with distilled water.

E. Mounting

- For DAB staining, perform dehydration and clearing with xylene after washing with water and then mount the sections using a water-insoluble mounting medium.
- For AEC staining, mount the sections using a water-soluble mounting medium after washing with water.

F. Dehydration and Clearing (needed only when water-insoluble mounting medium is used)

Table 2. Dehydration and Clearing Procedure

1	75% Ethanol bath	3 min	Dehydration
2	95% Ethanol bath	3 min	
3	100%(Absolute) Ethanol 1 bath	3 min	
4	100% (Absolute) Ethanol 2 bath	3 min	
5	Xylene 1 bath	3 min	Clearing
6	Xylene 2 bath	3 min	
7	Mounting with water-insoluble mounting medium		

Precautions for dehydration and clearing:

- Unless complete dehydration is achieved using absolute ethanol, a sample does not become clear after the clearing process. If it does not become clear, change to fresh solution and start again from the dehydration step.
- Prepare new reagents if a solution becomes dirty or if the alcohol concentration declines.
- Thoroughly remove the solution from the slides.
- Do not let the slides dry out during the dehydration and clearing processes.

VIII. Reference Information

1. Selection of Substrate, Mounting Medium, and Counterstaining Reagents

Enzyme	Substrate	Color	Mounting Medium	Characteristics
Peroxidase	DAB (3,3-Diaminobenzidine) * TaKaRa DAB Substrate (Cat. #MK210) is recommended.	Brown	Water-insoluble Mounting Medium	Dehydration and clearing is necessary. ● Slides can be stored semi-permanently. ● DAB preparation at the time of use is recommended. ● DAB is a carcinogen. Counterstaining: Hematoxylin (Blue) or Methyl Green (Green)
	AEC (3-Amino-9-ethylcorbazol)	Red	Water-soluble Mounting Medium	● Slides are not suitable for long-term preservation. ● AEC is stable in solution and can be preserved for a long period. Counterstaining: Hematoxylin (Blue)

2. Antigen Retrieval

When no information is available regarding the antigen retrieval method of the primary antibody, it is necessary to consider whether retrieval is necessary and which method should be used. The following heat treatment and proteinase treatment protocols are common antigen retrieval methods.

A. Heat Treatment

The following combinations of methods and buffers are most commonly used for antigen retrieval by heat treatment.

Methods :	Microwave Method Autoclave Method Hot Bath Method

Buffer :	Citric acid Buffer pH 6.0 Tris • EDTA Buffer pH 9.0

< Buffer preparation >

(1) Preparation of 10 mM sodium citrate buffer, pH 6.0
Add Solutions A and B to distilled water in the following ratio and mix thoroughly. (Prepare immediately before use.)

Solution A	9 ml
Solution B	41 ml
Purified water	450 ml
Total	500 ml

Solution A : 0.1 M Citric acid solution (Store at room temperature)
[2.1 g Citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$)/100 ml distilled water]
Solution B : 0.1 M Sodium citrate solution (Store at room temperature)
[14.7 g Trisodium citrate dihydrate ($C_6H_5O_7Na_3 \cdot 2H_2O$)/500 ml distilled water]

(2) Preparation of Tris• EDTA Buffer (TE) pH 9.0

Tris	1.21 g
EDTA • 2Na	0.37 g

Adjust to 1 L with distilled water

Mix well and adjust to pH 9.0. Add Tween 20 as needed to a concentration of less than 0.1% and mix thoroughly. This buffer can be stored at room temperature for 3 months or at 4℃ for a longer period of time.

< Methods of Antigen Retrieval using Heat Treatment >

＊Caution: Be careful not to burn yourself with buffers heated to high temperatures.

(1) Microwave method (500 W)

1) Heat the buffer to boiling by microwaving it in a heat resistant tray or a beaker (for about 5 min for 300 ml of buffer).
2) Soak the sections on slides in the buffer and mark the buffer level on the tray or beaker. Microwave for 5 min, taking care not to dry out the sections by evaporating the buffer. If buffer volume is reduced, add boiling purified water to the mark on the buffer tray (original liquid level).
3) Repeat Step 2 once or twice (for a total of 10 - 15 min).
4) While keeping the sections immersed in the buffer, remove the tray from the microwave. Allow the tray to cool slowly at room temperature for 20 min or more.
5) Wash 3X in PBS, for 3 min each at room temperature.

(2) Autoclave method

1) Add the buffer to a heat-resistant tray and soak the sections on slides in the buffer.
2) Autoclaving is performed at 120℃ for 20 min.
3) After pressure is reduced, remove the tray from the autoclave. Keeping the sections in the buffer, leave it for more than 20 min at room temperature and slowly cool it down.
4) Wash 3X in PBS, for 3 min each at room temperature.

(3) Hot bath method

1) Add the buffer into a heat-resistant tray and heat it in a hot bath at 95 - 99℃. Soak the sections on slides in the buffer and loosely cover the tray. The hot bath may also be loosely covered to maintain its temperature.＊
2) After making sure that the buffer temperature reaches 95 - 99℃ by using a thermometer, incubate slides for 40 min.
3) While keeping the sections immersed in the buffer, remove the tray from the hot bath. Allow the tray to cool slowly at room temperature for 20 min or more.
4) Wash 3X in PBS, for 3 min each at room temperature.

＊:An electric kettle may also be used to maintain the temperature at 98℃.

Note: Activation conditions for each antigen may differ depending on the heating method used. Some tissue sections may become detached because their shape changes. It is necessary to determine suitable conditions for the antigen by optimizing the heating time and choosing the best combinations of the above methods and buffers.

B. Proteinase Treatment

The enzymes used for proteinase treatment in antigen retrieval include protease, pepsin, and trypsin, etc. Because the extent of digestion by each enzyme is different, use the enzyme and digestion conditions recommended for your primary antibody. The standard conditions for each enzyme are shown below.

Enzyme	Temperature	Treatment time
0.05% Protease (Enzyme mix)	Room temperature	10 min
0.1% Proteinase K	Room temperature	3 - 6 min
0.4% Pepsin	37℃	20 - 30 min
0.1% Trypsin	37℃	30 min

(1) Remove any excess water around the sections.
(2) Cover the sections completely with proteinase solution and incubate using the appropriate temperature and treatment time.
(3) Wash the sections by soaking in PBS for 3 min.
(4) After changing PBS, wash the sections by soaking in fresh PBS for 3 min.
(5) Repeat Step 4 one more time (for a total of 3 PBS washes).

IX. Applications

1. Frozen Section Immunostaining

When frozen section immunostaining is performed using this product, perform a preliminary experiment according to the following guidelines:

- Frozen section preparation
  Tissue that is frozen after fixation should be sectioned. Unfixed frozen tissue (fresh frozen tissue) may not provide good staining results.
- Tissue fixative solution
  Since the choice of fixative solution depends on the primary antibody, select and use the appropriate fixative solution.
- Reaction time
  The recommended concentration and reaction time for the TaKaRa POD Conjugate is optimized for paraffin-embedded sections. If this product is used for frozen section staining, the protocol provided in this manual may increase background staining.
- Sample preparation
  When an antigen is unstable, a frozen section sample should be used. Tissue should be fixed using fixative solution (4% paraformaldehyde etc.) at 4℃ overnight, and frozen quickly with an water-soluble embedding medium (such as OCT compound embedding medium, etc.) using liquid nitrogen, dry ice-acetone, or dry ice-ethanol etc. Frozen sections should be sectioned to a thickness of 4 - 6 μm and attached to surface-treated slides (0.02% poly-L-lysine or silane). After drying, the embedding medium should be removed by washing with PBS.

＊ For necessary reagents, equipment, and instructions, refer to the protocol provided in this manual for paraffin-embedded sections.

2. Staining tissues from other species

A primary antibody derived from animals other than mice, rats, rabbits, and goats can be used with these products if there is cross-reactivity. In that case, a preliminary study is necessary to confirm whether or not a specific reaction takes place.

In addition, immunostaining of mini pig tissue can be peformed with the "For Tissue" products.

Primary antibody	Target tissue	Cat. #	Product name
Mouse	Mini pig	MK204	TaKaRa POD Conjugate Anti Mouse, For Tissue
Rabbit		MK205	TaKaRa POD Conjugate Anti Rabbit, For Tissue
Goat		MK203	TaKaRa POD Conjugate Anti Goat, For Tissue
Guinea pig	Mouse	MK202	TaKaRa POD Conjugate Anti Rabbit, For Mouse Tissue
	General	MK205	TaKaRa POD Conjugate Anti Rabbit, For Tissue

X. Analysis of Results

[Method]

Staining results should be observed with a light microscope. Samples slides should be evaluated in comparison to control slides.

- Positive control slides
  The presence of positive signals should be confirmed.
- Negative control slides
  Cells displaying positive signals should not be observed.
- Reagent control slides
  Cells displaying positive signals should not be observed. If cells on these slides display positive signals, this would provide evidence for a nonspecific reaction such as nonspecific protein binding, etc.

Notes:

- Sample slides are always evaluated compared to staining results of each control slide.
- It is important to remove embedding medium completely to obtain clear staining, because paraffin remnants cause background staining enhancement.
- In general, there are cases when false positive results are observed due to non-immunological binding of proteins and substrate reaction products. Sometimes false positive results are also caused by endogenous peroxidase reactions due to Cytochrome C or by false peroxidase reactions due to red blood cells.
- Necrotic tissue binds nonspecifically to antibodies and tends to cause nonspecific staining. Compare sample slides with negative control slides and evaluate results carefully.
- A portion of granulocytes and macropH ages contain Fc receptors on the surface of their cell membranes and are able to bind with the Fc site of the antibody used. There are times when staining is found elsewhere than the intrinsic specific reactive site of antibody used. Always compare sample slides with negative control slides and evaluate results carefully.

XI. Troubleshooting

Problem	Possible Cause	Solution
No staining or weak staining of positive control slides and sample slides.	Sections dried out during the staining process.	• Do not let tissue sections dry out during the staining process. Use a wet box.
	An inappropriate embedding medium was used, or paraffin removal from paraffin-embedded sections was incomplete.	• Select an appropriate embedding medium. • Remove paraffin completely from tissue. • Replace the xylene and ethanol solution with fresh solution.
	A very small amount of sodium azide in the buffer inactivated peroxidase and made staining impossible.	• Use a buffer that does not contain sodium azide. • Change the buffer or remake it.
	The enzyme did not react sufficiently with the antibody.	• Change the old substrate solution. • Remove extra solution completely at each step. • Make sure that the incubation time with the primary antibody was sufficient. It should be based on conditions shown to be optimal for that antibody.
Positive control slides are stained, but sample slides are not stained.	Antigens were fixed, denatured in the embedding process, or masked.	• Since some antigens are sensitive to fixation and embedding, use a mild fixative and shorten the fixation time. • In a case of masking, try an antigen retrieval by heat or proteinase treatment before staining.
	Antigens were destroyed by autolysis.	• Fix collected tissue immediately with an appropriate method.
	Antigens on the tissue were limited.	• Prolong the incubation time.
Background staining is strong on all slides.	Endogenous peroxidase is not inactivated.	• Ensure that treatment with 3% hydrogen peroxide in methanol is performed.
	There is nonspecific binding .	• Treat with 10% normal goat or rabbit serum before adding primary antibody.
	Insufficient treatment to inhibit endogenous mouse immunoglobulin (in the case of Cat. #MK200＊)	• Monitor the reaction time of Blocking Reagents A and B and make sure that the treatment is performed properly. • Bring Blocking Reagent A and B back to room temperature before use.
	Tissue fluid contains excess free antigens due to autolysis.	• Embed fresh tissue.
	Incomplete paraffin removal	• Change xylene and ethanol solutions.
	Insufficient antibody wash	• Wash antibody sufficiently.
	Enzyme reaction is too fast due to an excessively high room temperature.	• Control the room temperature so it falls within a standard range (15 - 5℃). • Shorten the reaction time.
	Sections dried out during the staining process.	• Do not let tissue sections that get wet in staining procedure dry out. Use a wet box.
Tissue sections are detached from slides during the reaction.	Antigen retrieval by heat or prolonged washing detached the tissue from the glass slides.	• Use a surface coating for tissue section slides, such as 0.02% poly-L-Lysine or silane. • Handle glass slides gently.

＊:Not available in all geographic locations. Check for availability in your area.

XII. Related Products

TaKaRa DAB Substrate (Cat. #MK210)
PH ospH ate Buffered Saline (PBS) Tablets, pH 7.4 (Cat. #T9181)
Proteinase K (Cat. #9034)