TOYOBO

COSMO BIO CO., LTD.
Inspiration for Life Science

Instruction manual Can Get Signal immunostain 0810

F0992K

Can Get Signal ® immunostain

Immunoreaction Enhancer Solution

NKB-401 5 ml x 2
NKB-501 20 ml
NKB-601 20 ml
Store at 4°C

Contents

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Inspiration for Life Science

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CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

[1] Introduction

Description

Can Get Signal® immunostain is a reaction solution that contains an accelerator for antigen-antibody reactions, which improves sensitivity, specificity, and S/N of immunohistochemistry (IHC) and immunocytochemistry.

Features

-Can Get Signal® immunostain improves sensitivity, specificity, and S/N of IHC.
-This system can be applied to various detection systems (*e.g.,* chromogenic, chemiluminescence, or fluorescence).
-This system can be used with ABC or polymer complex methods.
-Can Get Signal® immunostain consists of Solution A and B, which exhibit various properties for improving results. These reagents can be used independently.
-Reagents can be used directly without dilution. <Ready-to-use type>

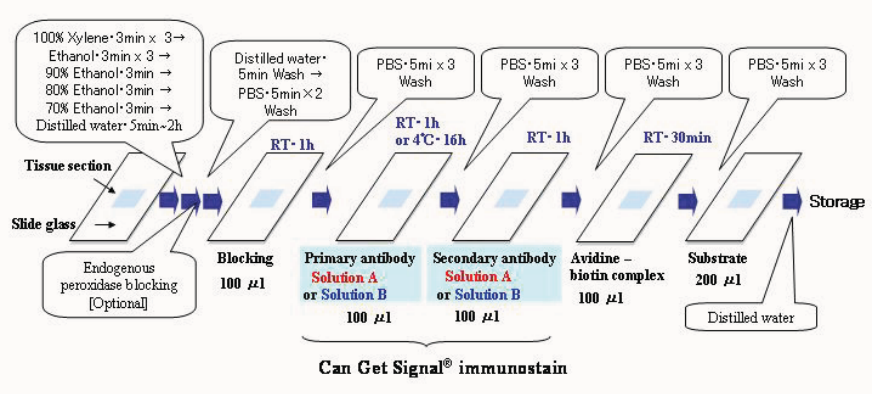

Fig. 1 Flow chart of ABC staining with IHC

Notes

-Can Get Signal® immunostain cannot be used as a blocking reagent. Blocking and detection steps should be performed using conventional methods.
-This reagent is not applicable to the avidin-biotin reaction in ABC method

[2] Components

This kit includes the following components. All reagents should be stored at 4°C and protected from light.

Reagent Name	Code No.		
	NKB-401	NKB-501	NKB-601
Solution A	5 ml	20 ml	-
Solution B	5 ml	-	20 ml

Notes

Can Get Signal® immunostain contains Solution A and B. These solutions exhibit various acceleration effects, which are antigen/antibody-dependent, and can be used independently. Both solutions should be examined prior to use.

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[3] Protocol

IHC is a method for detection of proteins located in tissue sections, which is accomplished through the use of antibodies that recognize target proteins. The antibody-antigen interaction is visualized by 1) chromogen detection, where an enzyme conjugated to an antibody cleaves a substrate to produce colored precipitate at the protein location, or 2) fluorescent detection, where a fluorophore is conjugated to an antibody and can be visualized using fluorescence microscopy.
The following is a protocol for IHC in fixed tissue (typically neutral buffered formalin), which is embedded in paraffin prior to sectioning, using the ABC method with HRP-conjugated antibodies. If secondary antibodies have been previously optimized, or for the polymer complex method (e.g. ENVISION+, Dako), refer to [3] 3.

1. Materials required

(1) Equipment:
- Hellendahl jar
- Slide
- Coverslip
- Mounting medium
- Marker pen

(2) Reagents and consumables:
-Ethanol
-Xylene
-PBS*
-Endogenous peroxidase blocking buffer*
-Blocking reagent*
-Chromogen substrate

*See [7] Reagent

2. Protocol for paraffin-embedded sections

(1) Place slides in rack and perform the following wash steps:
-Xylene: three times for 3 minutes each wash
-Ethanol: three times for 3 minutes each wash
-90% Ethanol: 3 minutes
-80% Ethanol: 3 minutes
-70% Ethanol: 3 minutes
-Distilled water: 5 minutes-2 hours

Notes

Formalin-fixed tissue sections often require an antigen retrieval step prior to IHC staining. During formalin fixation, methylene bridges between proteins are formed and antigenic sites become masked. Several antigen retrieval methods are effective for breaking the methylene bridges and exposing antigenic sites to allow antibodies to bind. Heat-mediated (or heat-induced) or enzymatic antigen retrieval method is generally sufficient.

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(2) **Optional:** Incubate in endogenous peroxidase blocking buffer for 30 minutes at room temperature in the dark.

Notes

Some cells or tissues contain endogenous peroxidase. Endogenous peroxidase activity, which may cause high background, can be significantly reduced by pre-treating cells or tissues with hydrogen peroxide prior to incubation with HRP-conjugated antibodies.

(3) **Optional:** Wash in distilled water for 5 minutes, and two times in PBS for 5 minutes.

(4) Dilute primary antibodies in Can Get Signal® Immunostain Solution A or B to an appropriate concentration.

Notes

Can Get Signal® Immunostain Solution A and B exhibit different acceleration effects, depending on antigens and antibodies. These solutions can be used independently; however, both solutions should be examined previously.

(5) After removing blocking reagent, add 100 µl diluted primary antibody solution and incubate at room temperature for 1 hour.

Notes

This reaction can be performed at 4°C overnight.

(6) Wash 3 times in PBS for 5 minutes.

(7) Dilute secondary antibodies in Can Get Signal® Immunostain Solution A or B to an appropriate concentration.

Notes

-Can Get Signal® Immunostain Solution A and B exhibit different acceleration effects, depending on antigens and antibodies. These solutions can be used independently; however, both solutions should be examined previously.

-Optimal antibody concentrations tend to be lower in this method than conventional methods. Therefore, antibody concentrations should be optimized based on lower concentrations.

(8) Subsequent to removal of primary antibody solution, add 100 µl diluted secondary antibody solution, and incubate at room temperature for 1 hour.

(9) Wash 3 times in PBS for 5 minutes.

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(10) After removing residual PBS, add 100 µl avidin-biotin complex solution and incubate at room temperature for 30 minutes.

Notes

The avidin-biotin complex solution should be used within 30 minutes after preparation.

(11) Wash 3 times in PBS for 5 minutes.

(12) After removing residual PBS, add 200 µl substrate solution and incubate at room temperature for an appropriate time.

(13) Rinse in distilled water to terminate the reaction.

(14) **Optional:** Counterstain.

(15) Mount coverslip with aqueous mounting medium or glycerol.

3. Protocol for paraffin-embedded sections using previously optimized secondary antibody concentrations or the polymer complex method

(1) Perform step (1)-(6) in [3] 2.

(2) After removing residual PBS, add 100 µl secondary antibody solution and incubate at room temperature for 30 minutes.

(3) Perform step (9)-(15) in [3] 2.

4. Protocol for frozen sections

(1) Wash the section 3 times in PBS for 10 minutes.

(2) Fix with the pre-cooled fixative (*e.g.,* acetone) for 5-10 minutes at room temperature.

(3) Wash in PBS for 10 minutes.

(4) Perform (2)-(15) in [3] 2.

NOTES

The use of paraffin-embedded sections with previously optimized secondary antibody concentrations, or the polymer complex method ([3] 3.), can be applied to this protocol.

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[4] Reagents

1. 10X PBS(-) (10X PBS) (500 ml)

5.75 g $Na_2HP0_4 \cdot 7H_20$
1.0 g KH_2PO_4
40.0 g NaCl
1.0 g KCl
Adjust volume to 500 ml

2. Endogenous peroxidase blocking solution (200 ml)

194 ml methanol
6 ml 10% H_2O_2

3. Blocking solution (10 ml)

10 ml 1X PBS(-)
150 µl normal serum

Notes

Animal species should be same between normal serum and secondary antibodies.

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[5] Troubleshooting

Symptom	Cause	Solution
High background/ Non-specific signal	Excessive primary antibody	In this method, optimal concentrations tend to be lower than conventional methods. Therefore, antibody optimization should be based on the lower concentrations.
	Excessive secondary antibody	-In this method, the optimal concentrations for secondary antibodies tend to be lower than conventional methods. Therefore, antibody optimization should be based on lower concentrations. -Previously optimized secondary antibodies can be diluted with this reagent.
	Insufficient blocking	-Prolong blocking time. -Change the blocking reagent.
	Insufficient washing	Increase wash steps or time.
	Endogenous peroxidase	-Prolong treatment time with endogenous peroxidase blocking buffer. -Increase H_2O_2 concentration of endogenous peroxidase blocking buffer up to 3%.
	Excessive exposure time (Fluorescent stain)	Decrease exposure time.
Weak signal	Insufficient primary antibody	Increase concentration of primary antibodies.
	Excessive blocking	- Optimize blocking time. - Change the blocking reagent
	Excessive washing	Decrease wash steps or time.
	Lack of antigenicity	-Tissue fixation method might be inappropriate. Change fixation method. -Antigen retrieval might be effective.
	Masking of antigenicity	Formalin-fixed tissue sections often require antigen retrieval prior to IHC staining.
	Antigen retrieval method is inappropriate	Optimize antigen retrieval conditions.
	Excessive exposure time (Fluorescent stain)	Decrease exposure time. Excessive excitation light bleaches fluorescence.

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