Nacalai USA
Innovations for Life Sciences.

Signal Enhancer HIKARI for Immunostain

Cat. No. NU00201 5 ml each
Cat. No. NU00202 20 ml
Cat. No. NU00203 20 ml

For Research Use Only

Introduction

Signal Enhancer HIKARI for Immunostain enhances antigen-antibody reactions. It can significantly enhance detection of weak immunoreactive and low abundance proteins in a variety of immunostain procedures such as immunohistochemistry (IHC) and immunocytochemistry. Simply dilute antibodies with Signal Enhancer HIKARI for Immunostain and process the rest of the procedures as usual. No additional steps are required. Signal enhancement is protein dependent and could vary from several folds to more than ten-fold.

Component

Description	Cat. No.	Quantity
Signal Enhancer HIKARI for Immunostain Starter Kit (Solution A & Solution B)	NU00201	5 ml each
Signal Enhancer HIKARI for Immunostain Solution A	NU00202	20 ml
Signal Enhancer HIKARI for Immunostain Solution B	NU00203	20 ml

Reagents Required but not provided

Equipment

- Hellendahl Jar
- Slide
- Coverslip
- Mounting medium
- Marker pen

Reagents and consumables

- Ethanol
- Xylene
- Phosphate-buffered saline (PBS)
- Endogenous peroxidase blocking buffer
- Blocking reagent
- Chromagen, fluorescent or chemiluminescent substrate

Storage

Upon receipt store kit at 4 °C and shielded from light.

A. Procedures for paraffine-embedded sections

1. Wash the paraffine-embedded slide with xylene, ethanol and water as in conventional preparation for IHC stain. [a,b]
2. Block the slide with a suitable blocking reagent at room temperature for 1 hour. Remove the blocking reagent. Wash three times with PBS for 5 minutes each.
3. Dilute primary antibody with Signal Enhancer HIKARI for Immunostain Solution A or B. [c] Optimize the dilution rate referring to the recommendation by the antibody supplier.
4. Add 100 μl diluted primary antibody solution and incubate at room temperature for 1 hour. (The incubation can be performed at 4°C overnight.) Remove primary antibody solution. Wash three times with PBS for 5 minutes each.
5. Dilute secondary antibody with Signal Enhancer HIKARI for Immunostain Solution A or B. [c] Optimize the dilution rate referring to the recommendation by the antibody supplier. [d,e]
6. Add 100 μl diluted secondary antibody solution and incubate at room temperature for 1 hour. Remove secondary antibody solution. Wash three times with PBS for 5 minutes each. [e]
7. Continue with an appropriate detection procedure to detect the protein of interest.

Notes

(a) 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 (ex. HistoVT One, Cat. No.: 06380-05) 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.

(b) 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.

(c) Signal Enhancer HIKARI for 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.

(d) Optimal secondary antibody concentrations tend to be lower using HIKARI than conventional methods. Therefore, antibody concentrations should be optimized based on the lower concentrations recommended.

(e) Skip step 5 when a polymer complex method or already optimized secondary antibody solution is used. Instead, add 100 μl diluted secondary antibody solution and shorten incubation time to 30 minutes in step 6.

B. Procedures for frozen sections

1. Wash the slide three times with PBS for 10 minutes each. Fix with the pre-cooled fixative (e.g., acetone) for 5-10 minutes at room temperature. Wash in PBS for 10 minutes.
2. Follow steps 2-7 in Procedures for Paraffin-Embedded Sections.

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Troubleshooting

Problem	Possible 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.

Technical Support

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