Can Get Signal® immunostain

Immunoreaction Enhancer Solution

(Code No. NKB-401, NKB-501, NKB-502, NKB-601, NKB-602)

Instruction Manual

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Caution

All reagents contained in this kit are for research purposes only. Never use them as reagents for diagnosis or other clinical purposes. Comply with general laboratory precautions and rules and ensure safety when using this kit.

[1] Introduction

***Can Get Signal*® immunostain** has been developed as a reaction-enhancing kit to address the problems often encountered during immunohistological staining and immunostaining of cells. These include inadequate sensitivity, low S/N ratio, and the appearance of nonspecific signals from secondary antibodies. In the past, inadequate detection sensitivity due to low antibody titers was dealt with by increasing the concentration of the added antibody. However, increasing the antibody concentration often caused background and nonspecific responses. By enhancing antigen-antibody reactions using this kit, it will improve the ability to optimize the antibody concentration, lead to clearer staining responses while suppressing nonspecific reactions caused by excessive addition of antibodies.

◆ Features ◆

1: Enhances immunostaining signal

***Can Get Signal*® immunostain** enhances antigen-antibody reactions, thus yielding higher signal compared with conventional techniques. With this kit it is possible to obtain images with a high S/N ratio and suppressed background signals for the detection of fluorescence or other light by reducing the amount of secondary antibody used or shortening the exposure time.

2: Applicable to a range of antibodies

***Can Get Signal*® immunostain** contains two solutions with different effects on antibodies (Solution A and Solution B). The reagent most suitable to a given experimental system can be selected. (When you first use this kit, it is recommended to use the Starter Set composed of both solutions, as described on the next page).

3: Applicable to a range of systems

None of the components of ***Can Get Signal*® immunostain** affect labeling enzymes or fluorescent dyes. The kit is therefore applicable to systems for detection of chemical coloration, chemiluminescence, and fluorescence. The kit can also be used in combination with sensitizing systems such as the ABC or polymer complex method.

4: User-friendly ready-to-use

***Can Get Signal*® immunostain** can be used without prior dilution.

[2] Composition

This product is made up of the following components.

Product and composition	Package	Code No.
***Can Get Signal*® immunostain** Starter Set Solution A Solution B	Each 5 ml	NKB-401
***Can Get Signal*® immunostain** Solution A	20 ml 20 ml x4	NKB-501 NKB-502
***Can Get Signal*® immunostain** Solution B	20 ml 20 ml x4	NKB-601 NKB-602

All reagents need to be stored protected from light at 4°C.

[3] How to use

(1) Antibody (primary antibody or labeled secondary antibody) is diluted with this kit and it is used directly for the assay. This kit is usually used to replace TBS (TBS-T), PBS (PBS-T) or blocking buffers for dilution of antibodies.

(2) This kit enhances antigen-antibody reactions, resulting in stronger signals than with conventional techniques. Due to an increase in reactivity, higher background signals may also occur. In such cases, the antibody concentration is probably not optimal, and **it will be difficult to obtain optimal staining responses using the same conditions as conventional techniques. To resolve this problem, it is advisable to review the concentration of the primary or secondary antibody used for detection or to readjust the signal detection time (exposure time) and other parameters.**

(3) **There are two types of reagents (Solution A and Solution B)** (see Previous Page for the composition of the kits). Solution A and Solution B have different effects on antibodies. The most suitable solution depends on the type of antibody, antigen, and sample involved. **It is advisable to try both solutions for a given assay system and select the solution that gives the best results.** A Starter Set composed of both Solution A and Solution B is available to select the more suitable of the two solutions (see Previous Page).

* Precautions *

- If a labeled secondary antibody or a polymer complex reagent is prepared in advance to an appropriate dilution for detection, this kit should be used only for dilution of the primary antibody. However, if the secondary antibody is to be diluted before use, this kit can be used as a diluent.

- This kit cannot be used for any step, such as the blocking reaction or avidin-biotin complex formation of ABC method, other than antigen-antibody reactions. This kit does not enhance the former reactions and, in some cases, the kit adversely affects them.

[4] How to stain paraffin sections

A method of peroxidase immunostaining using the ABC technique is presented below for reference. Various other immunostaining techniques are available, including methods using other enzymes or fluorescent dyes for labeling and methods using tyramide-based sensitization systems. ***Can Get Signal*® immunostain** is applicable to all such methods. If you plan to use secondary antibodies prepared in advance at an appropriate dilution or to use reagents for the polymer complex technique such as ENVISION+ (DAKO) and Histofine Simple Stain (Nichirei Corporation), you should also read **(4) Staining with a secondary antibody prepared in advance at an appropriate dilution**.

(1) Materials needed

- Staining bottle (Doze)
- Humidifying box
- Glass slide
- Marker pen for immunohistological staining
- Glass coverslip
- Timer
- Mounting agent

(2) Reagents needed

(*See [6] How to prepare reagents)

- Ethanol
- Xylene
- PBS*
- Endogenous peroxidase inactivating fluid*
- Blocking fluid*
- Substrate solution for color development (TMB, DAB, etc.)

(3) Staining procedure

1. Deparaffinize section in 2 changes of 100 % xylene for 5 min each, followed by hydration with ethanol.
2. Wash section with distilled water for at least 5 min.
3. If endogenous peroxidase inactivation is necessary, expose section to an endogenous peroxidase inactivating fluid for 10 min.
4. Wash section with distilled water for 5 min, followed by two changes of PBS, 5 min each.
5. Drop 100 μl of the blocking fluid onto the section and leave standing for 60 min to effect blocking.

6. After removing excess blocking fluid from the section, drop a solution of primary antibody, pre-diluted with Solution A or B of ***Can Get Signal***® **immunostain**, onto the section and leave standing for 60 min.
   - See **[3] How to Use** for details about the selection of Solution A or B and the antibody dilution ratio.
   - This reagent can also be used in combination with blocking agents such as BSA, casein or normal serum. Before diluting the antibody, these blocking agents need to be combined with this reagent.

7. Wash section with 3 changes of PBS, 5 min each.
8. After removing excess PBS from the section, drop a solution of secondary antibody, pre-diluted with Solution A or B of ***Can Get Signal***® **immunostain**, onto the section and leave standing for 60 min.
   - See **[3] How to Use** for details about the selection of Solution A or B and the antibody dilution ratio.
   - This reagent can also be used in combination with blocking agents such as BSA, casein or normal serum. Before diluting the antibody, these blocking agents need to be combined with this reagent.

9. Wash section with 3 changes of PBS, 5 min each.

10. After removing excess PBS from the section, drop 100 μl of an avidin-biotin complex solution onto the section and leave standing for 30 min. Prepare the avidin-biotin complex solution 30 min prior to use.

11. Wash section with 3 changes of PBS, 5 min each.

12. After removing excess PBS from the section, drop about 200 μl of a substrate solution onto the section to develop colors. Since the time required for color development varies greatly depending on the experimental system, the reaction needs to be monitored under a microscope and stopped at an appropriate time.

13. Immerse section in distilled water to stop reactions.

14. Mount section with glycerin or mounting agents and observe. Counterstain as needed. Dehydrate with ethanol and xylene and mount for observation.
    The appropriate mounting agent depends on the color developer used. Read the instructions carefully for both the color developer and the mounting agent you use.

(4) Staining with the secondary antibody prepared in advance at an appropriate dilution

If you plan to use secondary antibodies prepared in advance at an appropriate dilution, or to use reagents for the polymer complex technique such as ENVISION+ (DAKO Cytomation) and Histofine Simple Stain (Nichirei Corporation), you can only use this reagent for diluting the primary antibody.

1. Follow steps 1 through 7 in **(3) Staining procedure**.

2. After removing excess PBS from the section, drop 100 μl of the secondary antibody solution onto the section and leave standing for 30 min.

   You need to follow the instructions regarding the dilution, temperature, and time for the reagents used. If you plan to dilute the secondary antibody, you may use Solution A or B of ***Can Get Signal®*** **immunostain** as a diluent.

3. Follow step 9 and subsequent steps in **(3) Staining procedure**.

   You need to follow the instructions regarding washing and color development for each reagent used in these steps.

[5] How to stain frozen sections

1. Wash section with 3 changes of PBS, 10 min each.
2. Immediately before staining, fix the section using the method appropriate for the antigen used (such as acetone fixation).
3. Wash section with PBS for 10 min.
4. If endogenous peroxidase inactivation is required, follow Step 3 of **How to stain paraffin-embedded sections**.
5. Wash section with 3 changes of PBS, 5 min each.
6. Follow step 5 and subsequent steps in **[4] How to stain paraffin-embedded sections**.

[6] How to prepare reagents

・10x PBS(-) (10x PBS) (500 ml)

43 mM Na_2HPO_4・$7H_2O$ (MW = 268.07)

14 mM KH_2PO_4 (MW = 136.09)

27 mM KCl (MW = 74.55)

1.37 M NaCl (MW = 58.44)

Dissolve Na_2HPO_4 ・$7H_2O$ (5.75 g), KH_2PO_4 (1.0 g), NaCl (40.0 g) and KCl (1.0 g) in distilled water, to give a 10x solution (500 ml). Autoclave the solution as needed. The solution can be stored refrigerated or at room temperature.

If 10x PBS is refrigerated, sedimentation may occur due to the high salt concentration. If sediment forms, warm the PBS in a water bath (37°C), to completely dissolve the sediments before use.

Prepare 1x PBS by 1:10 dilution of 10x PBS with distilled water or sterile distilled water. Store the 1x PBS refrigerated or at room temperature.

- Endogenous peroxidase inactivating fluid (0.3% hydrogen peroxide/methanol; 200 ml)

  Add 10% Hydrogen peroxide (6 ml) to methanol (194 ml), and agitate to mix thoroughly. Prepare this fluid immediately before use. Do not add the methanol to the hydrogen peroxide. Generation of intense heat may occur.

- Blocking solution (1.5% normal serum/PBS; 10 ml)

  Add 150 μl normal goat serum to 10 ml of 1x PBS(-).

  If normal serum is used as a blocking agent, the serum is usually collected from animals of the same species as those from which the secondary antibody is derived.

[7] Trouble shooting

Problem	Cause	Countermeasure
High background or nonspecific staining responses	The use of this reagent has reduced the optimal concentration of the primary antibody.	Reduce the concentration of the primary antibody
	The concentration of the secondary antibody is too high.	Reduce the concentration of the secondary antibody. If you plan to use a secondary antibody prepared in advance at an appropriate dilution, dilute it with this reagent.
	Conditions for blocking are inappropriate	Review the blocking time as the length of blocking time can greatly affect the staining response. In some cases, changing the type of blocking agent may lead to favorable results.
	Washing is insufficient.	Increase the duration and frequency of washing.
	Endogenous peroxidase activity remains (if peroxidase-labeled antibody is used).	Extend the duration of the inactivating step or reduce the concentration of hydrogen peroxide to 3%.
	Exposure time is too long (for fluorescent staining)	Shorten the exposure time.
Weak signal	The concentration of the primary antibody is low.	Increase the antibody concentration.
	Blocking is excessive.	Review the blocking time as the length of blocking time can greatly affect the staining responses. In some cases, changing the type of blocking agent may lead to favorable results.
	Washing is excessive.	Reduce the duration and frequency of washing.
	Antigenicity has been lost.	Change the fixation method, as the problem may be associated with the fixation method used. In some cases, antigen potentiation may lead to favorable results.
	Antigen has been masked.	Unmask the antigen. Depending on the nature of the sample tested, the problem may be associated with the inability of the antibody to physically bind the antigen.

	Unmasking is inappropriate.	Review the unmasking conditions, since excessive unmasking can lead to loss of antigenicity.
	Exposure time is too long (fluorescent staining).	Shorten the exposure time, since too long an exposure to excitation can bleach the fluorescent dye.
Low stability of the mounted sample	The mounting agent is inappropriate.	Read the instructions for the color developer, fluorescent dye, and the mounting agent carefully. The appropriate mounting agent can vary depending on the color developer used and because the normal mounting agents for fluorescent dyes are not applicable to Alexa Fluor series fluorescent dyes.

[8] Related products

Product and composition	Package	Storage	Code No.	Price
Can Get Signal ® Immunoreaction Enhancer Solution (For Western blot and ELISA)				
Standard size (solutions for primary and secondary antibodies)	Each 250 ml	4°C	NKB-101	¥30,000
Trial size (solutions for primary and secondary antibodies)	Each 50 ml	4°C	NKB-101T	¥10,000
Solution for primary antibody only	250 ml	4°C	NKB-201	¥17,000
Solution for secondary antibody only	250 ml	4°C	NKB-301	¥17,000

◆In addition, Toyobo sells the products of the following antibody manufacturers.

See our on-line catalogue or each manufacturer's website for details.

On-line Catalogue, Life Science Division, Toyobo, Ltd.: **www.bio.toyobo.co.jp/bio01**

- **Santa Cruz Biotechnology, Inc. www.scbt.com**

  A wide range of antibodies, with emphasis on proteins involved in signal transduction. Reagents and kits for immunoassay are also available.

- **PeproTech, Inc. www.peprotech.com**

  Antibodies to growth factors and cytokines are available.

- **Fitzgerald Industries International, Inc. www.fitzgerald-fii.com**

  A wide range of antibodies is available including not only antibodies to cytokines and those involved in signal transduction but also antibodies to viruses, hormones, and narcotics.

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