Nacalai USA
Innovations for Life Sciences.

Signal Enhancer HIKARI

Cat. No. NU00101 50 ml
Cat. No. NU00102 250 ml

For Research Use Only

INTRODUCTION

Signal Enhancer HIKARI for Western Blotting and ELISA enhances antigen-antibody reactions. It can significantly enhance detection of weak immunoreactive and low abundance proteins in a variety of immunoassays such as Western blotting, dot blotting and ELISA. Simply dilute antibodies with **Signal Enhancer HIKARI** 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

Item Name	Cat. No.	Component	
Signal Enhancer HIKARI 50	NU00101	Solution 1	50 ml
		Solution 2	50 ml
Signal Enhancer HIKARI 250	NU00102	Solution 1	250 ml
		Solution 2	250 ml

Solution 1 for Primary Antibody
Solution 2 for Secondary Antibody

REAGENTS REQUIRED BUT NOT PROVIDED

- Tris-buffered saline (TBS) or phosphate-buffered saline (PBS)
- 0.05% Tween20 in TBS (TBS-T) or PBS (PBS-T)

STORAGE

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

A. PROCEDURES FOR WESTERN BLOTTING OR DOT BLOTTING

Important Note: For all steps, use sufficient volumes to completely immerse the blot.

1. For western blotting, separate proteins by electrophoresis and transfer proteins from the gel onto a nitrocellulose or PVDF membrane. For dot blotting, spot proteins directly onto a membrane without electrophoresis or transfer.
2. Block the membrane with a suitable blocking reagent.
3. Dilute primary antibody with **HIKARI Solution 1**. (If only one antibody is used, dilute the antibody with **HIKARI Solution 2** and skip step 4.) Optimize the dilution rate referring to the recommendation by the antibody supplier. Immerse the membrane in the diluted antibody solution and shake at room temperature for one hour. Wash with TBS-T (or PBS-T) three times.
4. Dilute secondary antibody with **HIKARI Solution 2**. Optimize the dilution rate referring to the recommendation by the antibody supplier. Immerse the membrane in the diluted antibody solution and shake at room temperature for one hour. Wash with TBS-T (or PBS-T) three times.
5. Continue with an appropriate detection procedure to detect the protein of interest.

B. PROCEDURES FOR ELISA (SANDWICH METHOD)

1. Prepare a 96-well ELISA plate with solid-phase antibodies.
2. Block the wells with a suitable blocking reagent.
3. Dilute antigen with **HIKARI Solution 1**. Optimize the dilution rate using serial dilution.
4. Dilute primary antibody with **HIKARI Solution 1**. Optimize the dilution rate referring to the recommendation by the antibody supplier.
5. Add antigen and primary antibody into each well and incubate at 37 °C for one hour. Wash with PBS-T three times.
6. Dilute secondary antibody with **HIKARI Solution 2**. Optimize the dilution rate referring to the recommendation by the antibody supplier.
7. Add secondary antibody to each well and incubate at 37 °C for one hour. Wash with PBS-T three times.
8. Continue with an appropriate detection procedure to detect the protein of interest.

TROUBLESHOOTING

WESTERN BLOTTING AND DOT BLOTTING

Problem	Possible Cause	Solution
Weak signals	Low protein concentration after electrophoresis	Use samples of as high concentration as possible in electrophoresis. Serial dilution of protein is useful in determining optimal concentration.
	Low antibody concentration	Determine optimal antibody concentration by dot blotting.
	Insufficient transfer to membrane	Increase electric current or transfer time. Usually the higher the gel concentration the lower the transfer efficiency. If a gradient gel is used, the difference in transfer efficiency between high-molecular weight and low-molecular weight proteins is increased. Efficiency may be improved by switching from semi-dry to wet transfer.
	Membrane transfer time too long and/or electric current too high	If using a nitrocellulose membrane, excessive transfer can cause protein to permeate across the membrane to the opposite side. Reduce electric current or shorten time in these cases. Changing to a PVDF membrane may also help.
Colorless band center	Antibody concentration too high	Depending on the detection reagent used, luminescence may be suppressed by excessive signals. Determine optimal antibody concentration by dot blotting.
Too many extra bands	Antibody concentration too high	Excessive antibody can increase nonspecific signals. Determine optimal antibody concentration by dot blotting.
	Protein concentration too high	Apply less concentrated protein in electrophoresis. Serial dilution is useful in determining optimal concentration.
	Insufficient blocking	Depending on the type of antigen and antibody, success or failure of blocking can depend greatly on the type and concentration of the blocking agent. Review the type and concentration of the blocking agent used.
	Insufficient washing	Increase frequency of washing.

ELISA

Problem	Possible Cause	Solution
Weak signals	Antigen or antibody concentration not high enough	Optimize antigen and antibody concentrations by serial titration.
Color too intense	Antigen or antibody concentration too high	Optimize antigen and antibody concentrations by serial titration.
	Duration of exposure too long	Shorten exposure time.
High background signals	Antigen or antibody concentration too high	Optimize antigen and antibody concentrations using serial titration.
	Insufficient blocking	Depending on the types of antigen and antibody, success or failure of blocking can depend greatly on the type and concentration of the blocking agent. Try different concentrations and/or different types of blocking agents.
	Insufficient washing	Increase frequency of washing.
Great variance in reading	Problem with the ELISA plate	Protein binding efficiency can vary greatly among different types of ELISA plate or among different batches of the same type of ELISA plate. When more accurate measurement is needed, select the ELISA plate carefully.

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