Cat. # T7111A

For Research Use

TaKaRa

Western BLoT Immuno Booster

Product Manual

v201912

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I. Description

Western BLoT Immuno Booster contains components that enhance antigen-antibody interactions. This product can be used in a variety of immunoassays, including Western blotting and ELISA, to increase the final detection sensitivity (varies from 4-fold to over 10-fold). This product does not effect the activity of horseradish peroxidase (HRP) or alkaline phosphatase (AP), and therefore is compatible with enzyme conjugated secondary antibodies and with any detection method (i.e., colorimetric or luminescence) .

To obtain the highest possible detection signal for Western blotting, use this product in combination with the Western BLoT HRP Substrate Series for HRP chemiluminescence.

II. Components

Western BLoT Immuno Booster Solution 1	250 ml
Western BLoT Immuno Booster Solution 2	250 ml

III. Storage

4℃

＊ Store at the recommended temperature and use within one year.

IV. Materials Required but not Provided

All reagents and equipment to perform the immunoassay (Western blot or ELISA). Using this product does not require any modifications to the assay protocol.

V. Precautions

These are precautions when using this product. **Please be sure to review prior to use.**

1. Use this product undiluted.
2. Western BLoT Immuno Booster Solution 1 is optimized for diluting a primary antibody, and Western BLoT Immuno Booster Solution 2 is for diluting a labeled secondary antibody. Solution 2 is also recommended for assays that use only one type of antibody, such as a labeled primary antibody.

VI. Protocol

1. **Antibody dilution:**
   Dilute the antibody to an appropriate concentration using Western BLoT Immuno Booster Solution 1 for primary antibodies and Western BLoT Immuno Booster Solution 2 for the labeled secondary antibody. Dilute at the same dilution ratio or a higher dilution ratio using ordinary dilution buffer (TBS-T or PBS-T). Depending on the assay system, we recommend performing a preliminary test to optimize the dilution ratio.

   For most assay systems and antibodies, Solution 2 is recommended for assays that use only one antibody, such as a labeled primary antibody.

2. **Immunoassay:**
   Perform immunoassays (e.g., Western blotting or ELISA) using the antibody diluted with this product. The assay may be performed according to the ordinary protocol.

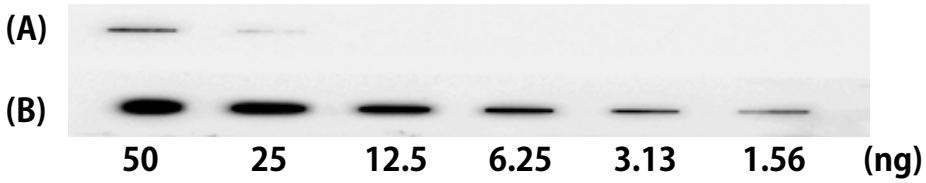
3. **Detection:**
   Use the ordinary detection method; no changes to the protocol are necessary when using this product. However, since this product improves detection sensitivity, the color development time or blot exposure time may need to be modified. Avoid long reaction times as they may lead to increased background.

VII. Experimental Example

Detection sensitivity was compared for antibodies diluted in either Western BLoT Immuno Booster Solution (B) or TBS-T containing 2% BSA (A).

Target:	Human transferrin (1.56 - 50 ng)
Assay:	Slot blot (nitrocellulose membrane)
Primary antibody:	Goat anti-human Transferrin, Polyclonal Antibody (1 mg/ml)
Secondary antibody:	Peroxidase labeled Rabbit anti-Goat IgG (0.5 mg/ml)
Detection substrate:	Western BLoT Quant HRP Substrate (Cat. #T7102A)
Detection:	CCD camera (exposure time 20 sec)

Dilution ratio	Antibody dilution buffer for (A)	Antibody dilution buffer for (B)
Primary: 1 : 2,000	2% BSA /TBS-T	Immuno Booster Solution 1
Secondary: 1 : 25,000	2% BSA /TBS-T	Immuno Booster Solution 2



Using the Western BLoT Immuno Booster for antibody dilution enhanced detection sensitivity by approximately 20-fold compared with using 2% BSA/TBS-T for dilution.

VIII. Troubleshooting

Since Western blotting and ELISA are multi-step processes, it may be necessary to optimize conditions at various steps in the protocol. We recommend preliminary tests to determine the appropriate quantity of protein sample, the optimum dilution ratios for primary and secondary antibodies, and other parameters.

■ Western blot

Problem	Cause	Solution
High background	The concentration of primary antibody used is too high.	Increase the dilution factor of the primary antibody to decrease the antibody concentration.
	Too much antigen has been used.	Reduce the quantity of antigen.
	Blocking is insufficient.	Optimize blocking conditions.
	The blocking reagent is unsuitable.	Use a different type of blocking reagent.
	Exposure time is too long (if detecting with X-ray film)	Shorten the exposure time.
	Washing is insufficient.	Increase the washing time, number of washes, or volume of wash buffer.
No band is visible or signal is weak	The primary antibody is unsuitable.	Confirm that the primary antibody recognizes the target protein, and the protein is not degraded.
	The type of secondary antibody is unsuitable.	Confirm that the secondary antibody recognizes the primary antibody and is not degraded.
	Quantities of antigen or antibody are insufficient.	Increase the quantity of antigen or antibody.
	Transfer of protein is insufficient.	Optimize transfer conditions.
	The film exposure time is too short (if using X-ray film for detection).	Increase the exposure time.

■ ELISA

Problem	Cause	Solution
High background	The concentration of primary antibody used is too high.	Increase the dilution factor of the primary antibody to decrease the antibody concentration.
	Too much antigen has been used.	Reduce the quantity of antigen.
	Blocking is insufficient.	Optimize blocking conditions.
	The blocking reagent is unsuitable.	Use a different type of blocking reagent.
	Washing is insufficient, or decreased blocking due to excessive washing.	Adjust the washing duration, number of washes, and/or the amount of washing buffer.
Weak signal	The amount of primary antibody is insufficient.	Use more primary antibody by decreasing the dilution factor.
	Quantities of antigen is insufficient.	Increase the amount of antigen.
Signal is too strong	Concentration of the antibody used is too high.	Use less primary antibody by increasing the dilution factor.
	Too much antigen was used.	Reduce the amount of antigen.
	The incubation time was too long.	Reduce the antibody incubation time.

IX. Related Products

＜ Western BLoT HRP Substrate Series ＞
Western BLoT Chemiluminescence HRP Substrate (Cat. #T7101A/B)
Western BLoT Quant HRP Substrate (Cat. #T7102A/B)
Western BLoT Hyper HRP Substrate (Cat. #T7103A/B)
Western BLoT Ultra Sensitive HRP Substrate (Cat. #T7104A/B)

＜ Western Blot Chemiluminescence Enhancer ＞
Western BLoT Immuno Booster PF (Cat. #T7115A)

＜ In place of a labeled secondary antibody ＞
Western BLoT Rapid Detect v2.0 (Cat. #T7122A)

＜ Blockng Buffer ＞
Western BLoT Blocking Buffer (Fish Gelatin) (Cat. #T7131A)
Western BLoT Blocking Buffer (Protein Free) (Cat. #T7132A)

＜ Stripping Buffer ＞
Western BLoT Stripping Buffer (Cat. #T7135A)

＜ Buffer Tablets and Powders ＞
Tris-Glycine-SDS Buffer (TG-SDS) Powder, pH 8.3 (Cat. #T9101)
Tris-Glycine Buffer (TG) Powder, pH 8.3 (Cat. #T9102)
Tris Buffered Saline (TBS) Tablets, pH 7.6 (Cat. #T9141)
Tris Buffered Saline with Tween20 (TBS-T) Tablets, pH 7.6 (Cat. #T9142)
Phosphate Buffered Saline (PBS) Tablets, pH 7.4 (Cat. #T9181)
Phosphate Buffered Saline (PBS) Tablets without Potassium, pH 7.4 (Cat. #T9182)
Phosphate Buffered Saline with Tween20 (PBS-T) Tablets, pH 7.4 (Cat. #T9183)

＜ Protein Ladder Marker ＞
CLEARLY Protein Ladder (Unstained) (Cat. #3453A/B)
CLEARLY Stained Protein Ladder (Cat. #3453A/B)

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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