Cat. # T7115A

For Research Use

TaKaRa

Western BLoT Immuno Booster PF

Product Manual

v201902

Table of Contents

I. Description

Western BLoT Immuno Booster PF contains components that enhance antigen-antibody interactions in immunoassays, such as Western blotting, to improve sensitivity and reduce background. This reagent is 100% chemically defined and completely protein-free. The product can be directly used to dilute primary and secondary antibodies and is effective in shortening reaction time, reducing the amount of antibody required, and improving detection of small amounts of antigen. This product does not affect 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. Because this product is for a protein-free detection system, it is recommended that the Western BLoT Blocking Buffer (Protein Free) (Cat. #T7132A) is used for blocking in Western blotting. Protein-free conditions minimize cross-reactivity that can occur with conventional protein-based blocking buffers, and are therefore ideal for detection of phosphorylated proteins and of low level antigens.

II. Component

Western BLoT Immuno Booster PF	250 ml

III. Materials Required but not Provided

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

IV. Storage

4℃
* Store at the recommended temperature and use within one year.

V. Precautions

The following are precautions for using this product. Read before use.

1. This product can be used as it is; do not dilute.
2. This product is a solution that can be directly used to dilute primary and secondary antibodies.
3. This product has been designed for use in protein-free conditions. Use together with a protein-free blocking reagent, such as Western BLoT Blocking Buffer (Protein Free) (Cat. #7132A)

VI. Protocol

1. Dilution of antibody:
   Dilute the antibody to an appropriate concentration using Western BLoT Immuno Booster Solution PF. Dilute at the same dilution ratio or a higher dilution ratio, as this product will increase sensitivity. Depending on the assay system, we recommend performing a preliminary test to optimize the dilution ratio.
2. Immunoassay:
   Perform immunoassays (e.g., Western blotting) 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 Examples

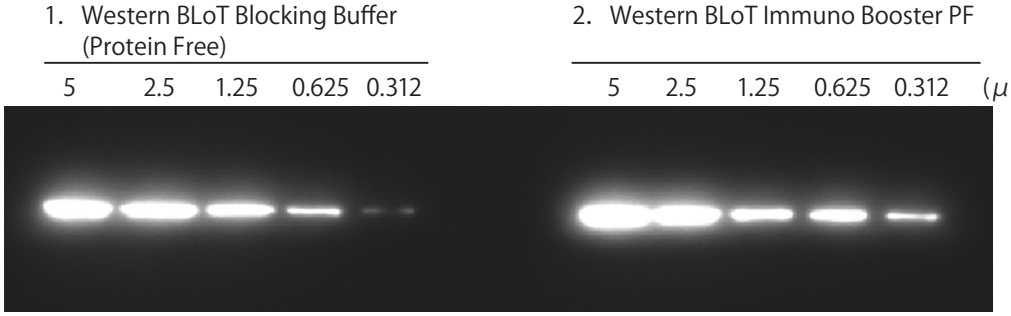
Example 1

Sample: HeLa cell lysate (5, 2.5, 1.25, 0.625, 0.312 μg/lane)
Blocking buffer: Western BLoT Blocking Buffer (Protein Free) (Cat. #T7132A)
Primary antibody: Monoclonal Anti-β-actin, clone AC-15 (2 mg/ml) [isotype IgG_1] (final concentration 0.5 μg/ml)
Secondary antibody: Goat Anti-Mouse IgG (H+L) Peroxidase conjugated (final concentration 0.16 μg/ml)

Antibody dilution:

1	Western BLoT Blocking Buffer (Protein Free)
2	Western BLoT Immuno Booster PF

Detection: Western BLoT Chemiluminescence HRP Substrate (Cat. #T7101A)
Wash buffer: TBS-T (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.6)



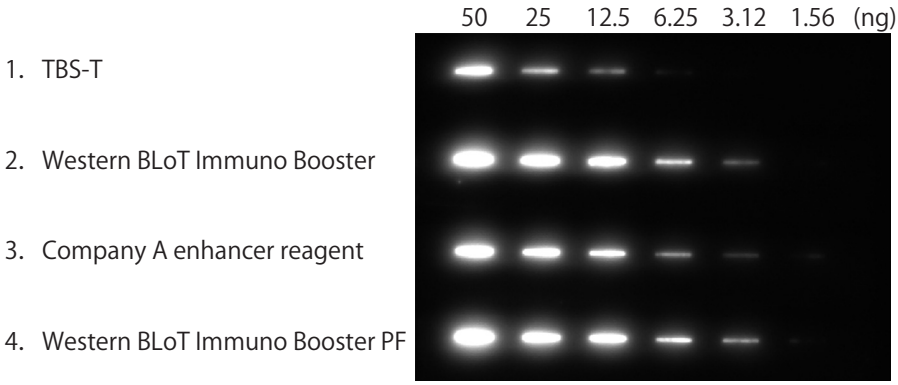
5 min exposure

Example 2

Sample: Human Transferrin（50, 25, 12.5, 6.25, 3.12, 1.56 ng/lane）
Blocking buffer: Western BLoT Blocking Buffer (Protein Free)（Cat. #T7132A）
Primary antibody: Goat anti-Human Transferrin affinity purified
（final concentration 0.5 μg/ml）
Secondary antibody: Affinity Purified Antibody Peroxidase Label Rabbit anti-Goat IgG (H+L)（final concentration 0.1 μg/ml）
Antibody dilution:

1	TBS-T
2	Western BLoT Immuno Booster（Cat. #T7111A）
3	Company A enhancer reagent
4	Western BLoT Immuno Booster PF

Detection: Western BLoT Chemiluminescence HRP Substrate
（Cat. #T7101A）
Wash buffer: TBS-T (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.6)



5 min exposure

VIII. Troubleshooting

Since Western blotting is a multi-step process, 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.

Problem	Cause	Solution
High background	The concentration of primary antibody 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.	The blocking reagent contains protein. **Use Western BLoT Blocking Buffer (Protein Free) (Cat. #T7132A).**
	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.

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 (Cat. #T7111A)
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 (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)