

Cat. # T7132A

For Research Use

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**TAKARA**

**Western BLoT Blocking Buffer  
(Protein Free)**

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Product Manual

v201902

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## I. Description

Selection of a blocking buffer is important to suppress non-specific signals and to obtain clear, specific Western blot results. The Western BLoT Blocking Buffer (Protein Free) is completely chemically defined and free protein-free reagent for blocking in Western blotting analysis. This product provides higher sensitivity than conventionally used blocking agents, such as nonfat milk. In addition to detection of general proteins, this blocking buffer is excellent for the detection of phosphorylated proteins.

In addition, completely protein-free assays are possible when this product is used in combination with Western BLoT Immuno Booster PF (Cat. #T7115A)\*.

- \* To obtain the highest detection sensitivity, combine with the Western BLoT HRP Substrate series.

## II. Components

Western BLoT Blocking Buffer (Protein Free) 500 ml

## III. Materials Required but not Provided

All reagents and equipment to perform Western blot analysis.

## IV. Storage 4°C

## V. Precautions

The following are precautions for using this product. [Read before use.](#)

1. This Blocking Buffer is ready-to-use without dilution for blocking Western blot membranes. Use directly for membrane blocking.
2. When a precipitate forms in the Blocking Buffer, use the Blocking Buffer only after the precipitate is completely dissolved by mixing at room temperature.

## VI. Protocol

This product is prepared in the optimal concentration for blocking; use it without dilution.

### Western blotting

1. Perform SDS-PAGE.
2. Transfer proteins from an SDS-PAGE gel to Western blot membrane, using an appropriate protocol.
3. Add Western BLoT Blocking Buffer (Protein Free) (use 0.2 ml/cm<sup>2</sup> membrane) to a tray of a suitable size, and immerse the protein-transferred membrane. Block the membrane for 1 hour at room temperature (20 - 30°C) with gentle shaking.
4. Remove the blocking buffer and perform Western blotting detection with appropriate antibodies. This product can also be used for dilution of primary and secondary antibodies.

## VII. Experimental Examples

### Example 1

Sample: HeLa cell lysate (5, 2.5, 1.25, 0.625, 0.312  $\mu$ g/lane)

Blocking buffer: Western BLoT Blocking Buffer (Protein Free)

Primary antibody: Monoclonal Anti- $\beta$ -actin, clone AC-15 (2 mg/ml)  
[isotype IgG<sub>1</sub>] (final concentration 0.5  $\mu$ g/ml)

Secondary antibody: Goat Anti-Mouse IgG (H+L) Peroxidase conjugated  
(final concentration 0.16  $\mu$ g/ml)

Antibody dilution:

1	Western BLoT Blocking Buffer (Protein Free)
2	Western BLoT Immuno Booster PF (Cat. #T7115A)

Detection: Western BLoT Chemiluminescence HRP Substrate (Cat.#T7101A)

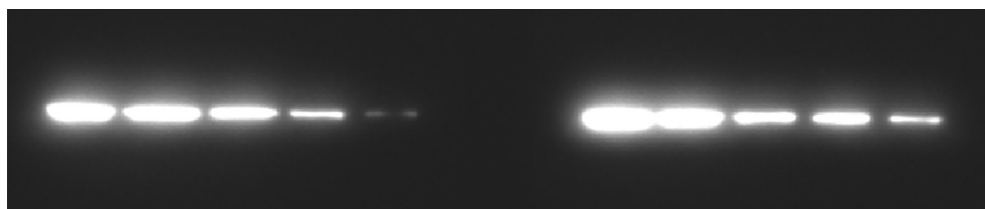
Wash buffer: TBS-T (10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.6)

1. Western BLoT Blocking Buffer  
(Protein Free)

5    2.5    1.25    0.625    0.312

2. Western BLoT Immuno Booster PF

5    2.5    1.25    0.625    0.312    ( $\mu$ g)



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)

Primary antibody: Goat anti-Human Transferrin affinity purified  
(final concentration 0.5  $\mu$ g/ml)

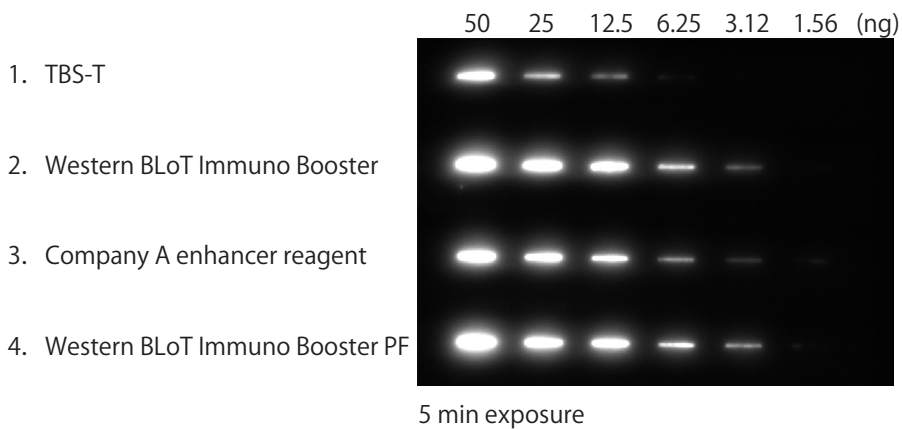
Secondary antibody: Affinity Purified Antibody Peroxidase Label Rabbit  
anti-Goat IgG (H+L) (final concentration 0.1  $\mu$ g/ml)

Antibody dilution:

1	TBS-T
2	Western BLoT Immuno Booster (Cat. #T7111A)
3	Company A enhancer reagent
4	Western BLoT Immuno Booster PF (Cat. #T7115A)

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)



## VIII. 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)
  
- < Blocking Buffer >
  - Western BLoT Blocking Buffer (Fish Gelatin) (Cat. #T7131A)
  
- < 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)

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**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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