

Cat. # T7132A

For Research Use

TAKARA

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

Product Manual

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I. Description

Selecting a blocking buffer that best suppresses non-specific signals is critical to obtaining clear, specific Western blot results. This product is completely chemically defined and free of protein components. Because of this, the Western BLoT Blocking Buffer (Protein Free) provides higher sensitivity than other commonly used blocking agents, such as nonfat milk. In addition to detection of normal 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 (contains preservative)

V. Precautions

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

1. This product is prepared at the optimum concentration for blocking.
2. When a precipitate forms, use the solution 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. Separate proteins by SDS-PAGE.
2. Transfer the protein to a PVDF membrane.
3. Add Western BLoT Blocking Buffer (Protein Free) (use 0.2 ml/cm² 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 proceed with Western blotting with the primary antibody. This blocking buffer can also be used to dilute the antibody.

VII. Experimental Examples

Example 1

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

Blocking buffer: Western BLoT Blocking Buffer (Protein Free)

Primary antibody: Monoclonal Anti- β -actin, clone AC-15 (2 mg/ml) [isotype IgG₁] (final concentration 0.5 μ g/ml)

Secondary antibody: Goat Anti-Mouse IgG (H+L) Peroxidase conjugated (final concentration 0.16 μ 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, pH7.6)

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 (μ 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 μ g/ml)

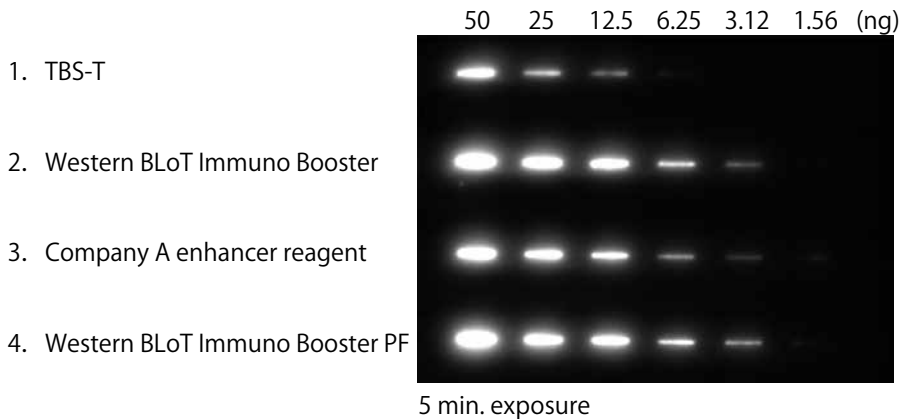
Secondary antibody: Affinity Purified Antibody Peroxidase Label Rabbit
anti-Goat IgG (H+L) (final concentration 0.1 μ g/ml)

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,
pH7.6)



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)

- < Buffers >
 - Tris-Glycine-SDS Buffer (TG-SDS) Powder, pH8.3 (Cat. #T9101)
 - Tris-Glycine Buffer (TG) Powder, pH8.3 (Cat. #T9102)
 - Tris Buffered Saline (TBS) Tablets, pH7.6 (Cat. #T9141)
 - Tris Buffered Saline with Tween20 (TBS-T) Tablets, pH7.6 (Cat. #T9142)
 - Phosphate Buffered Saline (PBS) Tablets, pH7.4 (Cat. #T9181)
 - Phosphate Buffered Saline (PBS) Tablets without Potassium, pH7.4 (Cat. #T9182)
 - Phosphate Buffered Saline with Tween20 (PBS-T) Tablets, pH7.4 (Cat. #T9183)
 - Western BLoT Blocking Buffer (Protein Free) (Cat. #T7132A)

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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