Cat. # T7135A

For Research Use

TaKaRa

Western BLoT Stripping Buffer

Product Manual

v201510Da

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I. Description

The Western BLoT Stripping Buffer is a reagent that can remove primary and secondary antibodies from Western blot membranes. After treatment with the Stripping Buffer, the membrane can be re-used; it is possible to probe the membrane with either a different concentration of primary antibody or with an entirely different primary antibody.

With this product, the antibody removal reaction proceeds under relatively mild conditions (room temperature, 30 minutes), and therefore there is very little loss of immobilized protein from the membrane. When using a PVDF membrane, the same membrane can be stripped and re-probed 2 - 5 times.

II. Components

Western BLoT Stripping Buffer	500 ml

III. Storage

Room temperature

IV. Materials Required but not Provided

Western blot membrane developed using chemiluminescence
- Western BLoT HRP Substrate series (Cat. #T7101A-T7104A) can be used for detection

Wash buffer (TBS or PBS containing 0.05% Tween 20)
- Tris Buffered Saline with Tween 20 (TBS-T) Tablets, pH7.6 (Cat. #T9142)
- Phosphate Buffered Saline with Tween 20 (PBS-T) Tablets, pH7.4 (Cat. #T9183)

Platform shaker

Reagents for Western blot detection (primary antibody, labeled secondary antibody, chemiluminescent detection reagent)

CCD camera or equipment and reagents for autoradiography.

V. Precautions

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

1. Use this product for membranes detected with a chemiluminescence. This product can not be used for membranes detected using chromogenic substrates such as TMB or DAB.
2. If the affinity of the antibody for the antigen is very strong, the antibody may not be completely stripped from the membrane.
3. Once the membrane has been used for detection, do not allow the membrane to dry. Store membranes submerged in PBS-T or TBS-T at 4℃ .
4. A white precipitate may form if the buffer is stored at a low temperature. In this case, warm in a water bath at 37℃ and shake gently to completely dissolve the precipitate before use.
5. This product is acidic. When using, wear a lab coat, safety goggles, and gloves.

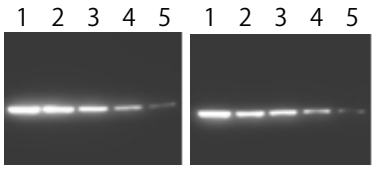
VI. Protocol

1. After chemiluminescent detection, wash the membrane in PBS-T or TBS-T buffer for 5 minutes. Discard the wash buffer.
2. Add Western BLoT Stripping Buffer so that the membrane is completely submerged. Shake gently for 15 - 30 minutes at room temperature. Use approximately 0.25 ml of the Stripping Buffer solution per cm^2 of membrane, and ensure that the membrane is completely immersed in the buffer during shaking.
3. Discard the Stripping Buffer. Wash the membrane for 5 minutes with PBS-T or TBS-T buffer.
4. Block the membrane and perform immunodetection using a new antibody.

VII. Experimental Example

PVDF membranes that were detected with Western BloT Chemiluminescence HRP Substrate (Cat. #T7101A) were stripped using the Western BLoT Stripping Buffer and re-probed. A reaction using TBS-T instead of the stripping buffer was included as a negative control.

1. β-actin detection (first blot)

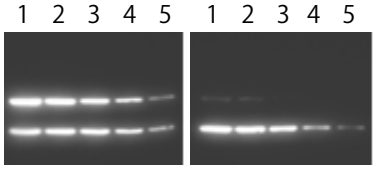


Lane
1： HeLa Cell Lysate 10.0 μg
2： HeLa Cell Lysate 5.0 μg
3： HeLa Cell Lysate 2.5 μg
4： HeLa Cell Lysate 1.25 μg
5： HeLa Cell Lysate 0.625 μg

Membrane:	PVDF membrane
Blocking:	Nonfat milk/TBS-T, room temperature, 60 min.
Wash buffer:	TBS-T
Primary Ab:	anti-β-actin (mouse IgG)
Secondary Ab:	anti-mouse IgG-HRP

Stripping (0.25 ml/cm^2 membrane, room temperature, 30 min.)

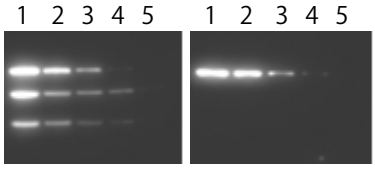
2. GAPDH detection (reprobe #1)



Wash buffer:	TBS-T
Primary Ab:	anti-GAPDH (mouse IgG)
Secondary Ab:	anti-mouse IgG-HRP

Stripping (0.25 ml/cm^2 membrane, room temperature, 30 min.)

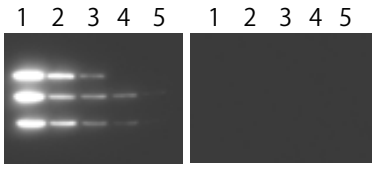
3. α-Tubulin detection (reprobe #2)



Wash buffer:	TBS-T
Primary Ab:	anti-α-Tubulin (mouse IgG)
Secondary Ab:	anti-mouse IgG-HRP

Stripping (0.25 ml/cm^2 membrane, room temperature, 30 min.)

4. Detection of residual antibody



Wash buffer:	TBS-T
Secondary Ab:	anti-mouse IgG-HRP

VIII. Related Products

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 Immuno Booster (Cat. #T7111A)
Western BLoT Immuno Booster PF (Cat. #T7115A)
Western BLoT Rapid Detect (Cat. #T7121A)
Tris-Glycine-SDS Buffer (TG-SDS) Powder, pH8.3 (Cat. #T9101)
Tris-Glycine Buffer (TG) Powder, pH8.3 (Cat. #T9102)
CLEARLY Protein Ladder (Unstained) (Cat. #3453A/B)
CLEARLY Stained Protein Ladder (Cat. #3454A/B)
Phosphate Buffered Saline with Tween20 (PBS-T) Tablets, pH7.4 (Cat. #T9183)
Tris Buffered Saline with Tween20 (TBS-T) Tablets, pH7.6 (Cat. #T9142)
Western BLoT Blocking Buffer (Fish Gelatin) (Cat. #T7131A)
Western BLoT Blocking Buffer (Protein Free) (Cat. #T7132A)