



Blot Restore
Membrane Rejuvenation Kit

Cat. No. 2520

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

The Blot Restore kit is an innovative tool for studies involving Western blot analysis.

It is widely believed that antigen-containing membranes used for Western blot experiments should never be dried. However, this is not true. The Blot Restore kit allows multiple re-use of membranes that were developed with substrate and subsequently dried. In model experiments both PVDF and nitrocellulose membranes showed excellent performance after been dried for at least two months.

Kit contains two components. Solution A re-hydrates dry membranes and washes out chemiluminescent developing reagents. Solution B strips both primary and secondary (labeled) antibodies off the membrane. Treated membranes are ready for blocking and subsequent re-probing with antibodies of choice.

Use of the Blot Restore Kit offers several advantages:

- 1) Conservation of samples that are expensive or available only in limited quantities,
- 2) Analysis of a given blot using several different antibodies, e.g. subtype- or isoform-specific antibodies,
- 3) Re-analysis of anomalous results and confirmation with the same or a different antibody,
- 4) Correcting errors in incubation with the wrong antibody,
- 5) Cost savings in reagents and time by reusing the same blot.

Application

The CHEMICON *Blot Restore* Kit is effective for hydrating dry membranes and removing antibodies from Western blots that have been developed with chemiluminescence or color substrates.

The *Blot Restore* Kit should be used only for qualitative purposes until it has been established by comparative blot analysis that stripping does not quantitatively affect a given antigen.

This product is for research use only; not for diagnostic or in vivo use.

Kit Components

1. **Blot Restore Solution A (10x)** - (1 container, 50 mL).
2. **Blot Restore Solution B (10x)** - (1 container, 50 mL).

Materials Not Supplied

- Standard Blot or blot strips, on nitrocellulose or PVDF/nylon membrane.
- Blocking Solutions.
- Distilled Water, for reagent dilution.
- Plastic Trays for incubation of blots or blot strips during hydration, stripping, washing and blocking solutions.

Storage

Kit components should be stored at 2-8°C upon arrival. Product is stable for 3 to 6 months after receipt.

Preparation of Reagents

1. Dilution of Blot Restore Solution A

Dilute Blot Restore Solution A 10x with distilled water to obtain a 1x solution. Prepare enough solution to allow free movement of strips or blots during incubation, typically 4 mL per strip or 10 mL per standard blot. Use the following chart for suggested volumes of Blot Restore Solution A.

# of Strips or Blots	Amount of 10x Blot Restore Solution A	Amount of Distilled Water	Resulting Amount of 1x Working Blot Restore Solution A
1 Strip	400 µL	3.6 mL	4 mL
5 Strips	1 mL	9.0 mL	10 mL
1 Blot	1 mL	9.0 mL	10 mL

2. Dilution of Blot Restore Solution B

Dilute Blot Restore Solution B 10x with distilled water to obtain a 1x solution. Prepare enough solution to allow free movement of strips or blots during incubation, typically 4 mL per strip or 10 mL per standard blot. Use the following chart for suggested volumes of Blot Restore Solution B.

# of Strips or Blots	Amount of 10x Blot Restore Solution B	Amount of Distilled Water	Resulting Amount of 1x Working Blot Restore Solution B
1 Strip	400 µL	3.6 mL	4 mL
5 Strips	1 mL	9.0 mL	10 mL
1 Blot	1 mL	9.0 mL	10 mL

Assay Protocol

Note: Immediately before use dilute Blot Restore Solution A and Solution B as described on page 3.

1. Fill plastic container with appropriate amount of Blot Restore Solution A (1x) solution.
2. Submerge membrane(s) in Blot Restore Solution A. Incubate with gentle agitation for 10 minutes at room temperature.
3. Discard Blot Restore Solution A and fill container with Blot Restore Solution B (1x) solution.
4. Incubate with gentle agitation for 15 minutes at room temperature.
5. Discard Blot Restore Solution B and fill container with your preferred blocking buffer (such as 20 mM TrisCl, 150 mM NaCl, 0.1% Tween 20, 5% dry milk or similar).
6. Incubate for 5 minutes at room temperature with gentle agitation.
7. Discard blocking buffer and fill the container with fresh portion of blocking buffer.
8. Incubate for 5 minutes at room temperature with gentle agitation.
9. Discard blocking buffer and proceed to incubation with your antibodies of choice.

Additional Usage Information

The Blot Restore system is designed for membrane “rejuvenation” with subsequent chemiluminescent detection. It will work with both membranes previously developed with chemiluminescent substrates and membranes previously developed with color substrate. However, it will also be applicable for situation when membrane is to be developed with color substrate different from substrate used before membrane was dried.

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