CHEMICON®
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Re-Blot
Western Blot Recycling Kit

Cat. No. 2060-S

Trial Size Kit

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Western blotting is a commonly used technique for studying protein function and localization. Typically, protein samples are electro-phoresed on SDS-PAGE and transferred to a membrane such as nitrocellulose or nylon, where they are probed with specific antibodies. Unlike nucleic acid based technologies, which allow reuse of Southern and Northern blots, it has been difficult to reuse Western blots.

Stripping and re-probing of Western blots 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.

While antigen and antibody-based immunoaffinity matrices, such as Sepharose conjugates, have been reused many times without compromising antigen-antibody reactivity, the need for pH extremes and chaotropic agents has precluded the application of these methods to Western blotting.

The CHEMICON *Re-Blot* Western Blot Recycling Kit contains specially formulated solutions that quickly and effectively remove antibodies from Western blots without significantly affecting the immobilized proteins.

Advantages of the *Re-Blot* Western Blot Recycling Kit include:

- No pungent-smelling β-mercaptoethanol is contained in the Antibody Stripping Solution.
- Antibody stripping is done at room temperature. No heating of blots is required.
- Blots can be stripped of antibodies in approximately 10 minutes at room temperature.
- Reblocking of blots may be avoided in most instances.
- Blots may be reused in less than 15 minutes.

Application

The CHEMICON *Re-Blot* Western Blot Recycling Kit is effective for removal of antibodies from Western blots that have been developed with chemiluminescence or radioactive iodine or other isotopes. It is not recommended for stripping colorimetric substrates (TMB, DAB, 4-chloronapthol, etc.), as it is not possible to effectively remove substrates that precipitate at the reaction site.

The *Re-Blot* Western Blot Recycling 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. **Antibody Stripping Solution (10x)** - (1 container, 10 mL).
2. **Blocking Buffer (20x)**; milk-based - (1 container, 10 mL).

Materials Not Supplied

- Standard Blot or blot strips, on nitrocellulose or PVDF/nylon membrane.
- Alternative Blocking Solutions, such as 10% BSA, for antibodies that are not compatible with milk-based blotting agents.
- Plastic Wrap, such as Saran Wrap, for storage of blots that will not be re-probed immediately.
- Distilled Water, for reagent dilution.
- Plastic Trays for incubation of blots or blot strips in stripping, washing and blocking solutions.
- Positive and Negative Stripping Controls. It is recommended that one new strip (not subjected to stripping solution) be included for comparison purposes.

Storage

Kit components should be stored at 2-8°C upon arrival. Product is stable for 3 to 6 months after receipt. If Antibody Stripping Solution crystallizes upon storage, it may be re-dissolved with gentle warming at 37°C before use.

Preparation of Reagents

1. Dilution of Antibody Stripping Solution

   Dilute Antibody Stripping Solution 10x with distilled water to obtain a 1x solution. If Antibody Stripping Solution contains crystals, warm gently at 37°C until crystals have dissolved completely. 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 stripping solution.

# of Strips or Blots	Amount of 10x Antibody Stripping Solution	Amount of Distilled Water	Resulting Amount of 1x Working Stripping Solution
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 Blocking Solution

   Dilute Blocking Solution 20x 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 blocking solution.

# of Strips or Blots	Amount of 20x Blocking Solution	Amount of Distilled Water	Resulting Amount of 1x Working Blocking Solution
1 Strip	200 µL	3.8 mL	4 mL
5 Strips	0.5 mL	9.5 mL	10 mL
1 Blot	0.5 mL	9.5 mL	10 mL

Assay Protocol

***Note**: The blots or individual strips that are to be re-used should be prepared for stripping immediately after their first usage. If stripping cannot be performed right away, membranes can be wrapped in plastic wrap and stored moist in PBS at 2-8°C. DO NOT STORE BLOTS IN DRY FORM.*

1. *Blot Stripping*. Fill plastic tray with appropriate amount of 1x Antibody Stripping Solution (see Preparation of Reagents for suggested quantities).
2. Using tweezers or forceps, submerge blot or blot strips in stripping solution. Incubate with gentle mixing for 10 to 15 minutes at room temperature.

   ***Note:** It may be necessary to increase the stripping incubation time when using blots that have been stripped previously. Simply increase the stripping time by 5 to 10 minutes, if needed.*
3. *Blocking*. Fill a clean plastic tray with an equal amount of 1x blocking buffer (see Preparation of Reagents for suggested quantities).
4. Wash/rinse blots two times 5 minutes with blocking buffer. It may be possible to use PBS or the primary antibody diluent for washing, as well.
5. The blot is now ready for reprobing with antibodies. It is suggested that users employ their own proven protocol and chemiluminescent detection method.

Additional Usage Information

Will membrane bound antigens be affected?

The proper use of the *Re-Blot* Western Blot Recycling Kit should not drastically affect membrane bound antigens. However, this kit should only be used for qualitative purposes, unless it has been established that stripping does not quantitatively affect a given antigen.

How many times can blots be stripped?

This will depend upon the nature of antigens being investigated. Most antigens should withstand the recycling protocol at least 5 times.

Are PVDF/nylon membranes compatible with this kit?

In general, nylon based membranes require either longer incubation or higher concentration of blocking agent (e.g., 10% BSA/milk instead of 5% for nitrocellulose). Alternate blocking conditions that are known to work with a given membrane can also be used.

Is it necessary to reblock blots that have been stripped previously?

It is generally not necessary to reblock with blocking solution after each stripping cycle. The blots can be kept moist in a sealed bag at 2-8°C for later use. However, if excessive background persists after stripping, blots can be reblocked with blocking buffer (diluted 1:20) or with other suitable buffers.

Can other blocking agents be used?

Some antibodies do not work very well with milk based blocking agents. If antibodies for reprobing are not compatible with the Blocking Solution supplied with the kit, a different agent that is known to be more effective with these antibodies may be substituted.

Blocking with certain agents (e.g., albumin) may lead to higher background upon stripping. Re-blocking with the supplied Blocking Solution may help reduce background.

Warranty

These products are warranted to perform as described in their labeling and in CHEMICON literature when used in accordance with their instructions. THERE ARE NO WARRANTIES WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND CHEMICON DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CHEMICON*'s* sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CHEMICON, to repair or replace the products. In no event shall CHEMICON be liable for any proximate, incidental or consequential damages in connection with the products.

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