CHEMICON®
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Re-Blot Plus
Western Blot Recycling Kit

Cat. No. 2500

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 Plus* 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 Plus* 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 15 minutes at room temperature.
- Blots may be reused in 25 minutes.

Application

The CHEMICON *Re-Blot Plus* 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 Plus* 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. **Mild Antibody Stripping Solution (10x)** - (1 container, 50 mL).

   (May be purchased separately as Catalog Number 2502.)

2. **Strong Antibody Stripping Solution (10x)** - (1 container, 50 mL).

   (May be purchased separately as Catalog Number 2504.)

Materials Not Supplied

- Standard Blot or blot strips, on nitrocellulose or PVDF/nylon membrane.
- Blocking Solutions.
- 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 4°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.

Note: To prevent reagent degradation secure the cap tightly upon storage. Avoid extended exposure to air.

Selection of Reagent

Re-Blot Plus Mild Stripping Solution gives good results on both nitrocellulose and PVDF membranes. However, *Re-Blot Plus* Strong Stripping Solution will perform better when membranes with high signal are to be stripped or when *Re-Blot Plus* Mild treatment is not sufficient.

Preparation of Reagents

1. Dilution of Antibody Stripping Solution

   IMMEDIATELY BEFORE USE:

   Dilute Antibody Stripping Solution (Mild or Strong) 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 20 mL per standard blot. Use the following chart for suggested volumes of stripping or blocking 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 (7 x 10 cm)	2 mL	18.0 mL	20 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 4°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 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 blocking buffer. Conventional blocking buffers such as 20 mM Tris HCl, pH 8.0; 150 mM NaCl; 0.1% Tween 20; 5% dry milk or similar are appropriate.
4. Wash blots two times 5 minutes each with blocking buffer.
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

To increase efficiency of the kit it is recommended to use minimal sufficient amounts of antigens transferred to the membranes. When several antigens are to be detected sequentially, it is recommended to start with antigens from which weaker signal is expected.

The proper use of the *Re-Blot Plus* 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. Most antigens should withstand at least 5 stripping cycles. However, it is to be taken into consideration that during each stripping cycle small portions of membrane-immobilized proteins will be removed.

References

Kawaguchi, S. and T. Hirano, (2002) Signaling cascade regulating long-term potential of $GABA_A$ receptor responsiveness in cerebellar purkinje neurons. *Journal of Neuroscience*. **22**:3969-3976.

Meertens, L. et al., (2004) A 10-amino acid domain within human T-cell leukemia virus type 1 and type 2 Tax protein sequences is responsible for their divergent subcellular distribution. *Journal of Biological Chemistry*. **279**:43307-43320.

Meertens, L. et al., (2004) Utilization of the CBP but not the p300 co-activator by human T-lymphotropic virus type-2 Tax for p53 inhibition. *Oncogene*. **23**:5447-5458.

Warranty

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August 2005
Revision D: 41061