Western Blot Recycling Kit Procedure
Total Stripping Time - ~ 15 min.

	The blots or individual strips that are to be re-used should be prepared for stripping immediately after their first usage. If stripping can not be performed right away, filters can be wrapped in Saran Wrap and stored moist in PBS at 4°C. DO NOT STORE BLOTS IN DRY FORM.
Step 1 *(Stripping)*	Dilute Antibody stripping solution with reagent quality water (1:10). This solution may crystallize but it will redissolve upon slight rewarming. Prepare enough solution to allow free movement of strips or blots during incubation. Incubate strips/blots with gentle mixing for 10-15 min at room temp.
	Note: Some antibodies may take a little more time to strip after each stripping cycle. Simply increase the stripping cycle to a few more min (e.g. 15-20 min).
Step 2 *(Blocking)*	Wash/rinse blots 2x with 1X blocking buffer (~5 min each). It may be possible to use PBS or the primary antibody diluent for washing as well.
	The Blot is now ready for reprobing with antibodies. It is suggested that the users employ their own proven protocol and chemiluminescent detection method. If not, we supply a complete chemiluminescence Western blot kit; Cat. No. 80200 or 80215).

Advantages of ADI's Western Blot Recycling Kit

- Antibody Strip solution has no pungent smelling mercaptoethanol.
- Antibody Stripping is done at room temp. No heating of blots is required.
- Strip antibodies in just about 10 min at room temp.
- Reblocking of blots may be avoided in most instances
- Reuse your blot in less than 15 minutes.

KIT PROFILE

Date received:_____ **Cat #** 90100 **Lot #**_____ **Exp.** _____
Dates used: _____
Dates used: _____
Remarks: _____

Instruction Manual No. M-90100-90102

Western Blot Recycling Kit

½ Cat. No. 90100
Cat. No. 90101
½ Cat. No. 90102

For Recycling Immunoblotting Membranes

ALPHA DIAGNOSTIC INTERNATIONAL

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Kit Contents:

(Sufficient reagents for 3000 cm^2 or 200-400 membrane strips or 20-40 standard blots)

Components	Cat.#
Antibody Stripping Solution (**10X**), 50 ml Before use, dilute 1:10 with water	**90101***
Blocking Buffer (**20X**), 50 ml; Milk-based Before use, dilute 1:20 with water	90102
Complete Instruction Manual	M-90100
***Note**: If item #90101 & #90102 are purchased separately then there will be **NO** other kit compoents supplied. Users may use their own blocking buffer or stripping buffers.	

INTRODUCTION

Western blotting is a commonly used technique to study protein structure and functions. Typically, protein samples are electrophoresed on SDS-PAGE and transferred to solid support (nitrocellulose or nylon-based membranes) for probing with antibodies. Unlike advances made in similar techniques in probing nucleic acids (northern and southern blotting) that allow reuse of blots, it has been difficult to reuse blots for Western blotting. Recycling of protein blots offers many advantages: 1) when protein samples are available in limited quantities, difficult to obtain or expensive, 2) when samples are to be analyzed with different antibodies under identical conditions, e.g., subtype or isoform specific antibodies, 3) when a blot gives unexpected results and needs confirmation with the same or a different antibody, 4) when a blot is mistakenly incubated with wrong antibody, 5) it is simply more economical and less time consuming to reuse the same blot. The idea of reusing blots derives strength from the fact antigen or antibody based immuno affinity matrices (e.g., antigen or antibody-linked Sepharose) can be easily reused many times by effectively disrupting the antigen-antibody interaction. However, the conditions (low or high pH, use of chaotropic agents) used in these technique have not been applicable to protein blots. ADI has now developed specially formulated solutions that effectively and almost quantitatively remove antibodies from the blots without significantly affecting the immobilized proteins.

PRECAUTIONS

The ADI Western blot recycling kit is for *in vitro research* use only.

STORAGE AND STABILITY

The reagents are stable at 4oC for 3-6 months or the specified expiration date.

General Guidelines and Suggestions

This kit is primarily designed to allow investigators to strip previously bound primary antibody and enzyme conjugates. All conditions for the optimum use of reagents should be optimized prior to attempting stripping and reuse. These conditions must remain the same after antibody stripping. All blots and strips that are to be reused should either be subjected to stripping immediately after their use or kept at 4°C moist in PBS in SaranWrap or a sealed bag. After stripping and reblocking, the blots can be incubated with new sets of antibodies or kept moist at 4°C for future use.

It is highly important to include a negative and a positive control to compare effective stripping. For example, one new strip (not subjected to stripping solution) should be included to assure and compare proper working of all reagents and antibodies after stripping. The proper use of recycling kit should not drastically affect the membrane bound antigens. However, this kit should only be used for qualitative purposes unless it has been established that recycling does not quantitatively affect a given antigen. 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 4°C for later use. However, if excessive background persist after stripping, we recommend that blots be reblocked with blocking buffer (diluted 1:10) or with other suitable buffers.

This kit has only been optimized to be used with ADI's chemiluminescent kit (Cat. 80200 & 80210). Chemiluminescent substrate kit from other vendors should work as well. This kit can be used to strip antibodies that were labeled with radioactive Iodine or other isotopes. **It is not recommended for procedures that use color development (TMB, 4-chloronaphtol, etc) as it is not possible to completely remove the substrate that precipitates at the reaction site.**

How many times blots can be stripped? this will depend upon the nature of antigens being investigated. Most antigens should be able to withstand the recycling protocol up to 5-8 times or more.

Use of PVDF/nylon membranes with this kit? Nylon based membranes in general require either longer incubation or higher concentration of blocking agent (e.g., 10% BSA/milk instead of 5% with nitrocellulose). Therefore, blocking conditions that are known to work with a given membrane can be used after stripping.

Use of blocking agents other than that in the kit? Some antibodies do not work very well with milk based blocking agents. It is suggested that another blocking agent that is known to work with a given antibody be tried after each stripping. Blocking with certain agents (e.g., albumin) may lead to higher background upon stripping. Re-blocking with the supplied blocking buffer may help reduce background.

Citations of ADI's Western blot Kit #90100 (See updated publication list at the web site)

McClelland GB et al **2003,** Biochemical and Biophysical Research Communications 304, 130-155, **Used PVDF, stripped and reprobed with various MCTs antibodies**

Jackson, S K. **2002**, Immunology 106 Issue 4 Page 486, **stripped adn probed with actin**

Zhao L 2002, Molecular Endocrinology 16(12):2902–2912, PVDF

Penttinen C, **2002**, J Cell Sci. 115, 3457-3468 used NC

Humphreys RC et al **2002**, Endocrinology Vol. 143, No. 9, 3641-3650; **Used Nitrocellulose; Stripped 4 times and reprobed with other antibodies**

Dwivedi PP **2002,** J. Biol. Chem., Vol. 277, Issue 33, 29643-29653 , **Used Nitrocellulose, Stripped phosphoantibodies and reprobed with other antibodies**

McClelland GB et al **2002** , J Appl Physiol, Apr 2002; 92: 1573-**1584 Used PVDF, stripped and reprobed with various MCTs antibodies**

Jackson, Stephanie K. **2001**, J Immunol 166, 952-958; **Used Nitrocell; primary antibody stripped and re-probed with beta-actin antibodies**

Lorenzo, Patricia S. **2001,** Cancer Res. 2001 61: 943-949, **Used Nitrocellulose, Stripped ERK1/2 phosphoantibodies and reprobed with other antibodies**