Strip and reprobe western blots

Introduction

Following western blot detection using a chemiluminescent or fluorescent substrate system, researchers often wish to strip off the first set of protein probes (e.g., primary and secondary antibodies, avidin–biotin complex) so that different proteins on the blot may be detected using a second set of specific probes. When possible, this technique saves the time and resources that would be necessary to electrophorese another sample and transfer it to a new sheet of membrane.

Stripping generally involves soaking the blot in a buffer that is sufficiently harsh to dissociate the affinity interactions between antibody or other probes and the target protein that was transferred to the membrane. The goal is to find a stripping condition that is efficient in removing the probes without also removing or damaging the target proteins on the blot. Because every antibody–antigen affinity interaction and target protein is unique, no one stripping condition is appropriate for all situations. Empirical testing and some optimization will be necessary to determine appropriate stripping conditions for a particular western blot system. In most cases, conditions can be optimized to make at least one round of stripping and reprobing possible; in some cases, several rounds of stripping and reprobing are possible.

Be aware that stripping conditions will not dissociate avidin–biotin or streptavidin–biotin affinity interactions (because these interactions, though noncovalent, are very strong), although the intact complex may be stripped from the target to which it is bound. For example, it is generally not possible to strip a streptavidin–HRP probe from biotinylated cell surface proteins that have been electrophoresed and transferred to a membrane. On the other hand, a complex of a biotinylated primary antibody and streptavidin–HRP may be stripped from the target protein on the membrane.

Traditional stripping buffers

A simple, mild stripping buffer is 0.1 M glycine-HCl (pH 2.5–3.0). Commonly used for elution in affinity purification methods, this buffer will dissociate most antibody–antigen interactions in less than 30 min at room temperature (RT) or 37°C. In some cases, incubation for 2 hr may be necessary. In other cases, this buffer will not effectively remove antibodies from blots.

A frequently used, relatively harsh stripping buffer formulation is 50 mM Tris-HCl (pH 7) with 2% sodium dodecyl sulfate (SDS) and 50 mM dithiothreitol (DTT) [1]. Effective stripping with this buffer usually requires incubation for 30 min at 70°C; some denaturation and loss of target protein is inevitable. Since DTT is unstable, this buffer must be prepared immediately before each use.

Restore western blot stripping buffers

Thermo Scientific™ Restore™ Western Blot Stripping Buffer (Cat. No. 21059) and Restore™ PLUS Western Blot Stripping Buffer (Cat. No. 46430) provide generally robust but gentle formulations for stripping primary and secondary antibodies from blots to enable several reprobings on the same membrane. They are ready-to-use, odorless formulations that combine low pH (2.8) and special additives to efficiently dissociate probe interactions on nitrocellulose (NC) or polyvinylidene fluoride (PVDF) membranes. In most cases, blots are stripped effectively when incubated in a Restore buffer for 5–15 min at 37°C or RT. After washing the blot in a neutral buffer (e.g., PBS or TBS), stripped blots may be tested for complete removal of primary and secondary probes or simply reprobed with new primary and secondary antibodies.

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Available western blot stripping buffers.

	Restore stripping buffer	Restore PLUS stripping buffer	Restore fluorescent stripping buffer
Features	• Gentle, odor free	• Robust yet gentle, odor free • Designed for use with antibodies that are difficult to remove from western blots, require longer incubation times, or incubation temperatures greater than 22°C	• Gentle and highly effective reagent for quickly removing primary and NIR dye–labeled secondary antibodies from western blots
Membrane	NC and PVDF	NC and PVDF	Use with low-fluorescence PVDF membranes (e.g., Cat. No. 22860)
Incubation	5–15 min at 37°C	5–15 min at RT or 37°C for high-affinity antibodies	10–20 min at RT
Select when …	… primary antibody is susceptible to stripping buffers	… removing high-affinity primary antibodies	… removing NIR-labeled antibodies

Traditional stripping buffers may not be effective for removing dye-labeled secondary antibodies, which may then overwhelm the target signal when reprobing. Thermo Scientific™ Restore™ Fluorescent Western Blot Stripping Buffer (Cat. No. 62300) is a gentle and highly effective reagent for quickly removing primary and near-infrared (NIR) dye–labeled secondary antibodies (680–800 nm) from western blots. It is for use with Thermo Scientific™ Low-Fluorescence PVDF Transfer Membrane (Cat. No. 22860).

Consult the product instructions for detailed information about Restore, Restore PLUS, and Restore Fluorescent Western Blot Stripping Buffers, as well as protocols for testing stripping efficiency.

Reference

1. Harlow E, Lane D, editors (1999) Using Antibodies: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Ordering information

Product	Quantity	Cat. No.
Restore Western Blot Stripping Buffer	500 mL	21059
Restore PLUS Western Blot Stripping Buffer	500 mL	46430
Restore Fluorescent Western Blot Stripping Buffer	100 mL	62300
Low-Fluorescence PVDF Transfer Membrane, 0.2 µm, 7 cm x 8.4 cm	100 sheets	22860

Find out more at **thermofisher.com/strippingbuffers**

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