

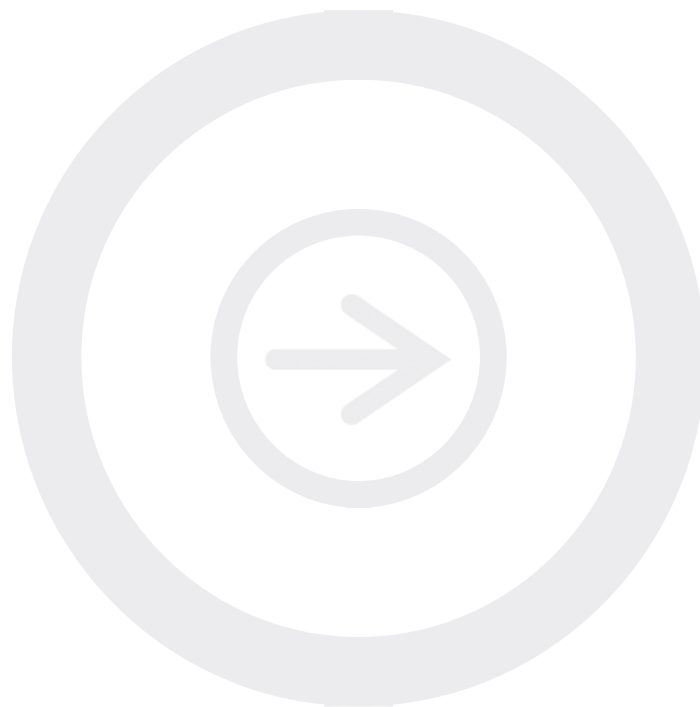
NewBlot™ Nitrocellulose 5X Stripping Buffer

Developed for:

Odyssey® Family of Imagers

Please refer to your manual to confirm that this protocol is appropriate for the applications compatible with your model of Odyssey Imager.

Part Number: 928-40030
Pack Size: 100 ml
Storage: Room temperature



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Biosciences

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Table of Contents

	Page
I. Stripping Guidelines	3
II. Procedure	4
III. Optimization Guide	4-6
IV. Frequently Asked Questions	7

Reagents Provided

NewBlot Nitrocellulose 5X Stripping Buffer, 100 ml (sufficient for up to 3000 cm² or approximately fifty 7 x 8.5 cm Odyssey® nitrocellulose membranes)

Reagents Needed

- 1X PBS wash buffer (LI-COR, P/N 928-40018, 10X PBS)
- Ultrapure water
- LI-COR Incubation Box - Large (LI-COR, P/N 929-97301) or equivalent
- Completed Western blot to be stripped (wet)

I. Stripping Guidelines

- Familiarize yourself with the entire *Procedure*, *Optimization Guide*, and *Frequently Asked Questions* before proceeding. Ensure that you have the necessary supplies and reagents for stripping the membrane.
- Always prepare fresh working solution for each blot to be stripped. The working solution of stripping buffer should only be reused when a blot requires an extended amount of time to remove lingering fluorescent signal (after the initial stripping step). NewBlot Nitrocellulose is provided as a (5X) concentrated solution.
- Do not allow the membrane to dry out at any point during the Western assay or the stripping process. If you must leave the membrane unattended between steps, store it in 1X PBS buffer.
- NewBlot Nitrocellulose Stripping Buffer has been optimized for use with LI-COR IRDye® antibody conjugates and Odyssey nitrocellulose membrane.
- Western blots probed with IRDye 800CW, IRDye 680LT, or IRDye 680 conjugates can be effectively stripped using NewBlot Nitrocellulose Stripping Buffer. NewBlot Nitrocellulose should not be used with IRDye 700DX conjugates. NewBlot Nitrocellulose may perform well with nitrocellulose membranes or antibody-dye conjugates from other manufacturers, but LI-COR neither guarantees nor supports such use.

II. Procedure

1.	Prepare a 1X working solution of NewBlot Nitrocellulose Stripping Buffer by mixing one part NewBlot with four parts water. The large LI-COR® incubation box (11.75 x 8.9 cm) with a 7 x 8.5 cm membrane requires a minimum of 20 ml. Optimization of the working solution concentration may be required.
2.	Transfer working solution of NewBlot Nitrocellulose Stripping Buffer to a clean incubation container.
3.	Place blot to be stripped into the incubation container. Make sure the blot is fully submerged and can move freely within the container. Place container onto shaker/rotator and allow to shake briskly (70-80 rpm) for 5 minutes at room temperature.
4.	Immediately rinse blot by removing from stripping buffer and placing into a fresh container of 1X PBS; use enough PBS to completely submerge the blot. Repeat rinse two more times in succession using fresh 1X PBS each time (a total of 3 rinses). Note: Allow blot to remain in 1X PBS after the last rinse until further processing. Do not allow the blot to dry.
5.	Image the blot on an instrument from the Odyssey® family of imagers to ensure complete removal of sample fluorescence.
6.	If sample fluorescence remains, refer to <i>Optimization Guide</i> . Otherwise, proceed to reprobe the blot with the desired primary and subsequent secondary antibodies.

III. Optimization Guide

The three main factors which affect stripping efficacy on Odyssey nitrocellulose membranes are time, concentration, and temperature. If there remains significant signal on the blot after completing the steps in *Procedure* above, continue to strip the blot as follows, until desired results are achieved:

1. Place the blot back into the 1X working solution of NewBlot Nitrocellulose Stripping Buffer. Place container onto shaker/rotator and shake briskly for 5 minutes (room temperature).
2. Immediately rinse by removing blot from stripping buffer and placing into a fresh container of 1X PBS. Repeat rinse two more times in succession using fresh 1X PBS each time.
3. Image the blot on an instrument from the Odyssey family of imagers to ensure complete antibody removal.
4. If fluorescent signal remains, prepare a 2X working solution of NewBlot Nitrocellulose Stripping Buffer and repeat steps 1-3. This process can be repeated by increasing the Stripping Buffer concentration incrementally (up to 5X) until signal removal is satisfactory.

Note: NewBlot Nitrocellulose Stripping Buffer is provided as a '5X' concentrated solution.

Increasing the stripping time and the stripping buffer concentration will progressively reduce the ability to successfully reprobe the blot due to the increased likelihood of damage to the target antigen. Following the preceding optimization guidelines in step-wise fashion will help minimize this damage while improving chances of successful fluorescent signal removal. In some cases, fluorescent signal cannot be removed completely, even under the most stringent stripping conditions. This is due to a number of factors, including sample load amount, antibody affinity/avidity, and target protein abundance. One factor which can greatly reduce the ability to successfully strip and reprobe a blot is whether or not the blot was allowed to dry at all during the incubation, washing, scanning, and stripping steps. ***Make certain that the blot is kept moist at all times.***

Stripping time has the greatest effect on stripping efficiency, followed by stripping buffer concentration. Increasing the temperature also significantly improves stripping effectiveness, but can also have a highly detrimental effect on reprobing. If the optimization process given here does not produce the desired stripping results, Stripping Buffer incubation can be carried out at 37°C using a water bath or warm-air incubator. ***Do not microwave the NewBlot Nitrocellulose Stripping Buffer or the nitrocellulose blot.***

The example data shown in the following figures illustrate the relative strip and reprobe effectiveness for incubation time, Stripping Buffer concentration, and temperature. The sample and protein targets, in this case, were not sufficiently stripped using the standard stripping Procedure; thus, optimization was required.

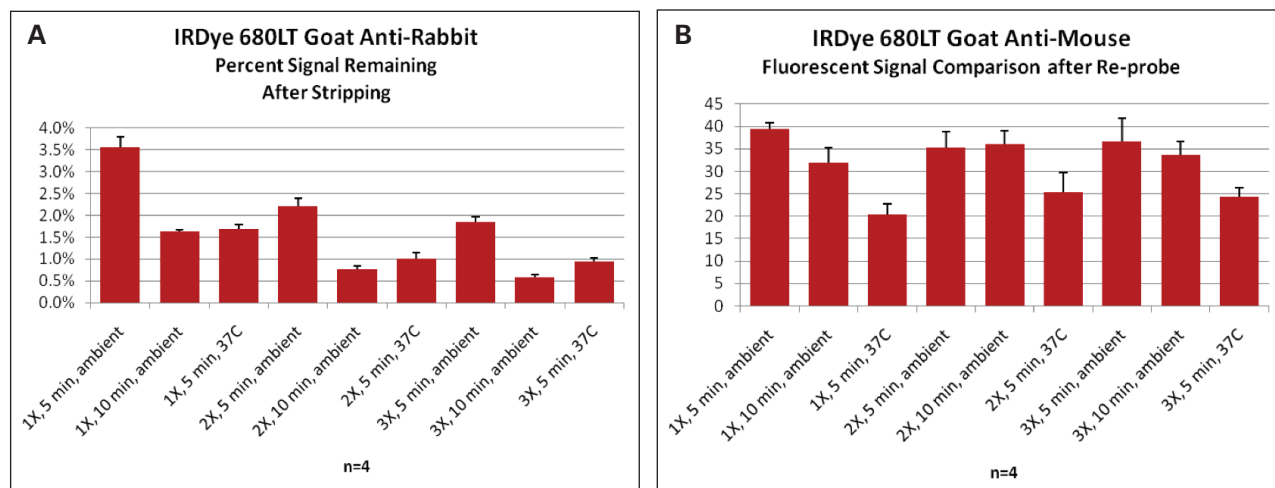


Figure 1. (A) Relative stripping effectiveness of β -Actin and IRDye® 680LT goat anti-rabbit antibodies when varying Stripping Buffer concentration, incubation time, and incubation temperature. Quantitation of internal replicates is represented here as a percentage of total fluorescent signal remaining on the blots after stripping. (B) Fluorescent signal quantitation after reprobing the stripped blots with a different set of primary and IRDye 680LT secondary antibodies.

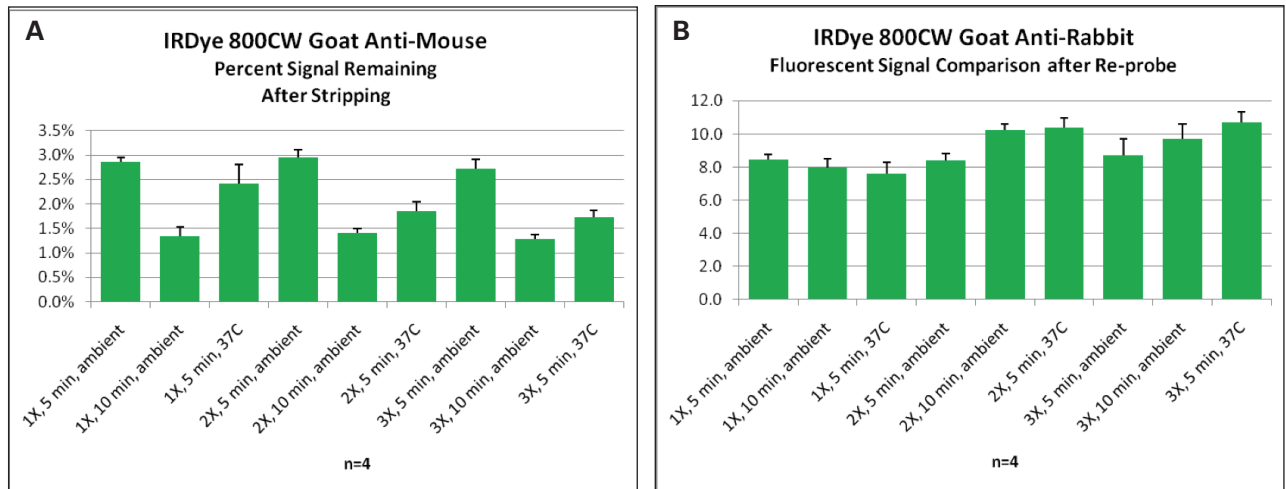


Figure 2. (A) Relative stripping effectiveness of EGFR and IRDye 800CW labeled goat anti-mouse antibodies when varying Stripping Buffer concentration, incubation time, and incubation temperature. Quantitation of internal replicates is represented here as a percentage of total fluorescent signal remaining on the blots after stripping. (B) Fluorescent signal quantitation after reprobing the stripped blots with a different set of primary and IRDye 800CW labeled secondary antibodies.

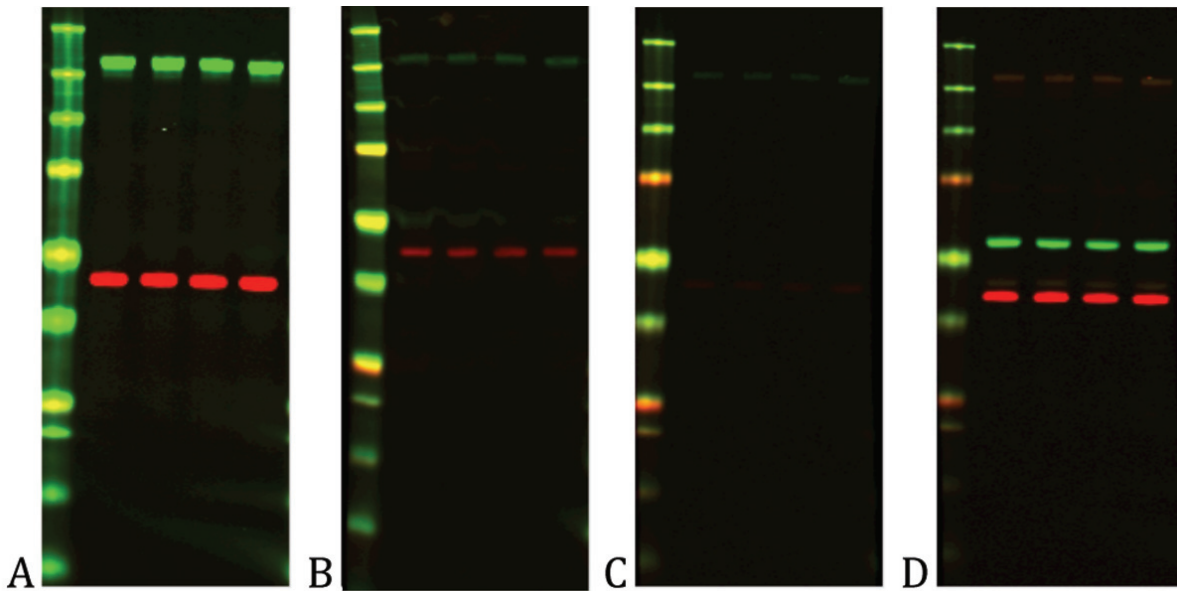


Figure 3. Images showing an example of Western blot stripping optimization. (A) Initial Western blot, showing EGFR detected with IRDye 800CW goat anti-mouse and β-Actin detected with IRDye 680LT goat anti-rabbit. (B) After stripping with standard stripping Procedure. (C) After stripping optimization – in this case, 3X concentrated Stripping Buffer for 10 min at ambient temperature. (D) After reprobe with new set of antibodies.

IV. Frequently Asked Questions

How many times can a membrane be stripped?

Typically, up to three times. The number of times is dependent on several factors, including the type and amount of bound antigen, the type of membrane used, and the stringency of stripping conditions.

Can I quantify after stripping and reprobing?

Quantification can be performed to evaluate band intensities within the reprobbed blot, but not to compare band intensities from previous analyses of the same blot. Only qualitative analysis should be performed when comparing stripped/reprobbed blots.

Can I reuse the stripping solution?

The presence of IRDye® infrared dye in used stripping solution will contribute to high background fluorescence when reprobing the membrane; therefore, only the use of freshly-prepared stripping solution is recommended.

Why do I see high background fluorescence after stripping and reprobing my membrane?

Typically, reblocking the membrane after stripping is not necessary. If you see an excessive amount of background signal, however, additional blocking may help. Block for 15-30 minutes in Odyssey® Blocking Buffer prior to reprobing your membrane.

Why am I still seeing bands even after stripping for an extended period of time? Or, why can I not see any bands after reprobing my blot?

There are many factors that affect the overall performance of the NewBlot Nitrocellulose Stripping Buffer. The concentration of Stripping Reagent, as well as incubation time, can be adjusted up or down to best suit your samples. Please refer to the *Optimization Guide* for more information.

Can I use the NewBlot Stripping Buffer for blots probed with alternate dye-labeled antibodies?

NewBlot Stripping Buffer has been used with a number of fluorescently labeled antibodies from other manufacturers, and has worked quite well in most cases; however, NewBlot Stripping Buffer is optimized for use with LI-COR® IRDye 800CW, IRDye 680LT, and IRDye 680 secondary antibodies. NewBlot Nitrocellulose Stripping Buffer cannot be used with IRDye 700DX secondary antibodies.

Will NewBlot Nitrocellulose work with chemiluminescent/colorimetric substrates?

NewBlot Stripping Buffer is not formulated for use with chemiluminescence detection or colorimetric substrates; therefore, it is only recommended for use with LI-COR fluorescently labeled secondary antibodies.

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