

# NewBlot™ PVDF 5X Stripping Buffer

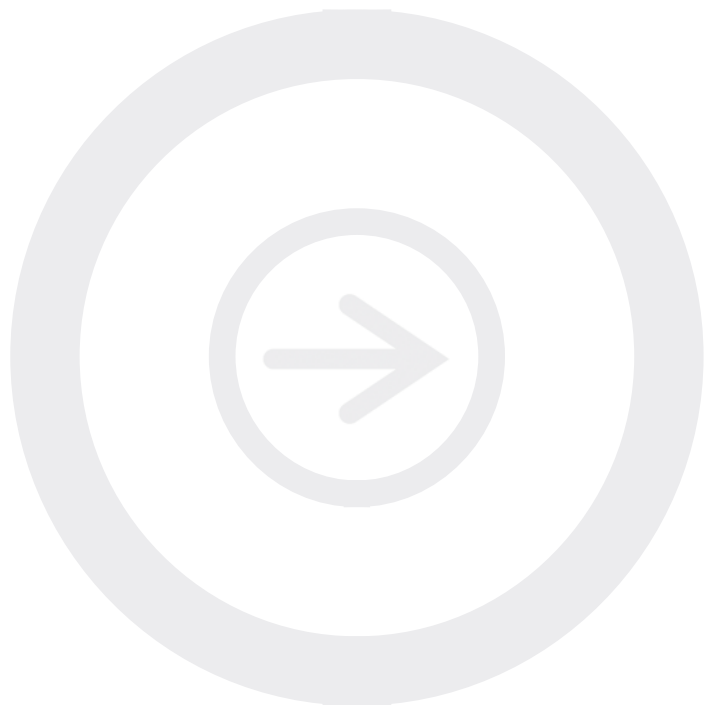
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Developed for:

## 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: 928-40032  
Pack Size: 100 ml  
Storage: Room temperature



**LI-COR®**  
Biosciences

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## Reagents Provided

NewBlot PVDF 5X Stripping Buffer, 100 ml (sufficient for up to 3000 cm<sup>2</sup> or up to fifty 7 x 8.5 cm Millipore Immobilon®-FL PVDF membranes)

## Reagents Needed

- 1X PBS wash buffer (LI-COR, P/N 928-40018, 10X PBS)
- Ultrapure water
- 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 PVDF Stripping Buffer is provided as a (5X) concentrated solution.
- Do not allow the membrane to become dry 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 PVDF Stripping Buffer has been optimized for use with LI-COR IRDye® antibody conjugates and Millipore Immobilon-FL PVDF membrane.
- Western blots probed with IRDye 800CW, IRDye 680LT, or IRDye 680 conjugates can be effectively stripped using NewBlot Stripping Buffer. NewBlot Stripping Buffer should ***not*** be used with IRDye 700DX conjugates. NewBlot Stripping Buffer 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 by mixing one part NewBlot PVDF 5X Stripping buffer 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 volume of 20 ml. Optimization of the working solution concentration may be required.
2.	Transfer working solution of NewBlot PVDF 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 20 minutes at room temperature.
4.	<p>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 this step two more times in succession using fresh 1X PBS each time (a total of 3 rinses).</p> <p><b>Note:</b> Allow blot to remain in 1X PBS after the last rinse until further processing. Do not allow the blot to dry.</p>
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 PVDF membranes are time, temperature, and inclusion of SDS detergent. If there remains significant signal on the blot after completing the *Procedure* above, continue to strip the blot as follows, until the desired results are achieved:

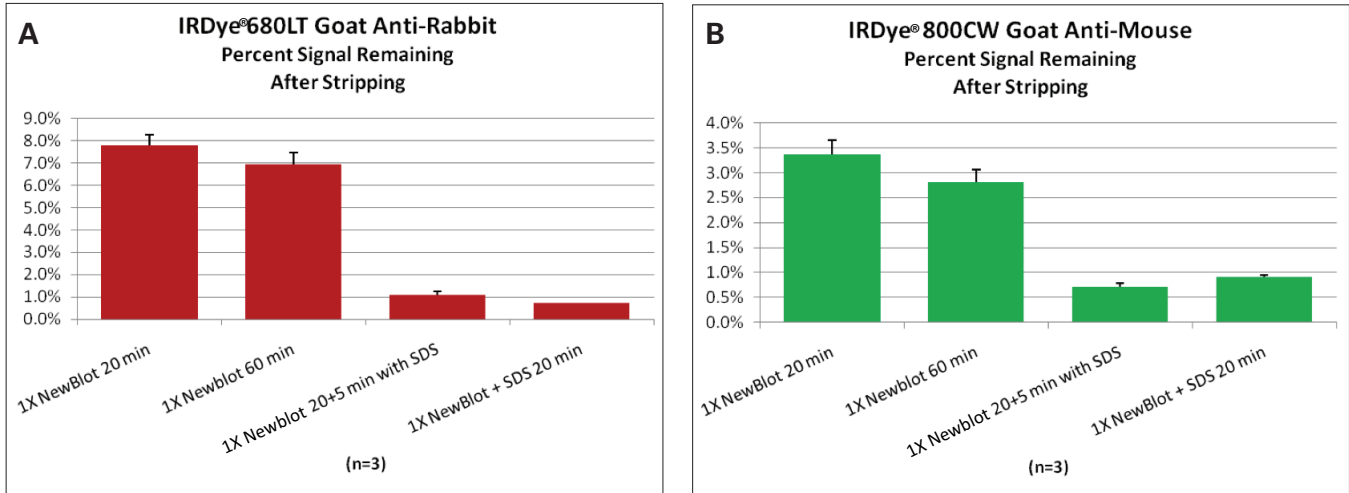
1. Place the blot back into the 1X working solution of NewBlot PVDF Stripping Buffer. Place container onto shaker/rotator and allow to shake briskly for 10 minutes (room temperature).
2. Immediately rinse blot by removing from stripping buffer and placing into a fresh container of 1X PBS. Repeat rinse 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, add Sodium Dodecyl Sulfate (SDS) to the Stripping Buffer container, to a final concentration of 0.5% (v/v), and mix. Place blot into the 1X Buffer + SDS solution and shake briskly for 5 minutes (room temperature).
5. 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.
6. Image the blot on an instrument from the Odyssey family of imagers to ensure complete antibody removal.
7. If fluorescent signal remains at this point, incubation time can be extended in increments of 5 or 10 min, until desired results are achieved.

*Optional: Incubation in Stripping Buffer can be carried out at 37°C using a water bath or warm-air incubator. This should only be attempted if the above optimization steps are unsuccessful, since heating during the stripping process will significantly reduce the ability to successfully reprobe the blot. Do not allow the solution or the membrane to overheat.*

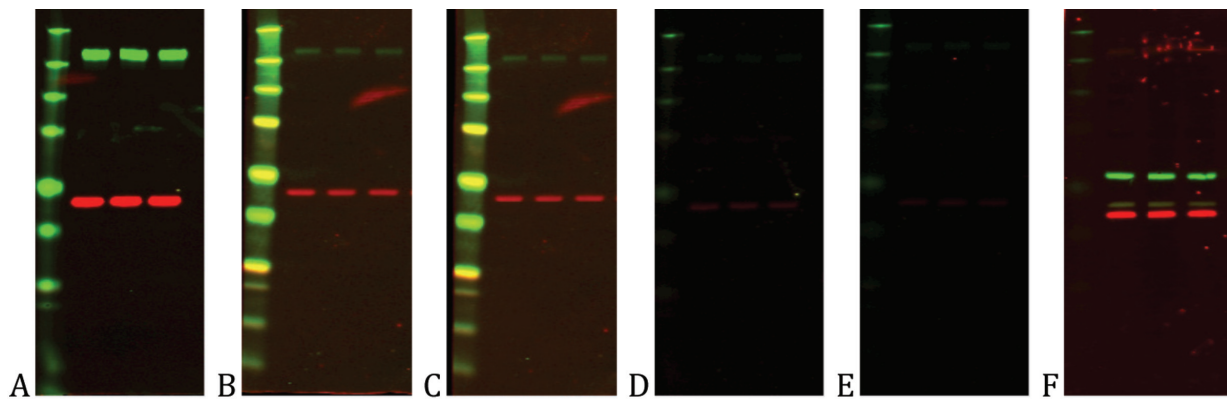
Increasing the stripping time and temperature, and adding SDS to the stripping solution will progressively reduce the ability to successfully reprobe the blot due to the increased likelihood of damage to the target antigen. Following the preceding optimization guidelines in step-wise fashion will help to minimize this damage, while improving the 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 several 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.***

The example data shown in the following figures illustrate the relative strip-and-reprobe effectiveness of increasing time and addition of SDS. The sample and protein targets, in this case, were not sufficiently stripped using the standard stripping *Procedure*, so optimization was required.



**Figure 1.** Relative stripping effectiveness on (A)  $\beta$ -Actin and IRDye® 680LT goat anti-rabbit antibodies, and (B) EGFR and IRDye 800CW goat anti-mouse antibodies with different Stripping Buffer conditions: (from left) 1X NewBlot for 20 min (standard conditions), 1X NewBlot for 60 min; 1X NewBlot for 20 min, followed by an additional 5 min incubation after the addition of SDS; 1X NewBlot + SDS for 20 min.



**Figure 2.** Images showing an example of Western blot stripping optimization with NewBlot PVDF Stripping Buffer. (A) Initial Western blot, showing EGFR detected with IRDye 800CW goat anti-mouse and  $\beta$ -Actin detected with IRDye 680LT goat anti-rabbit. (B) After stripping with standard stripping *Procedure*. (C) After stripping for an additional 20 min. (D) After additional 5 min stripping with the addition of SDS. (E) After 20 minutes total stripping time in NewBlot PVDF + SDS. (F) Reprobe image showing ERK2 detected with IRDye 680LT goat anti-mouse and  $\beta$ -Tubulin detected with IRDye 800CW goat anti-rabbit.

## 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 reprobated blot, but not to compare band intensities from previous analyses of the same blot. Only qualitative analysis should be performed when comparing stripped/reprobed blots.

### ***Can I reuse the stripping solution?***

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

### ***Why do I see high background fluorescence after stripping and reprobating 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 reprobating 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 reprobating my blot?***

There are many factors that affect the overall performance of the NewBlot PVDF Stripping Buffer. Stripping Buffer incubation time and temperature can be increased, or SDS can be added to the stripping buffer to enhance the stringency of the buffer. Please refer to *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 works quite well in most cases; however, NewBlot Stripping Buffer is optimized for use with LI-COR® IRDye 800CW, IRDye 680LT or IRDye 680 secondary antibodies. NewBlot PVDF Stripping Buffer cannot be used with IRDye 700DX secondary antibodies.

### ***Will NewBlot PVDF work with chemiluminescent/colorimetric substrates?***

NewBlot Stripping Buffer has not been tested 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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