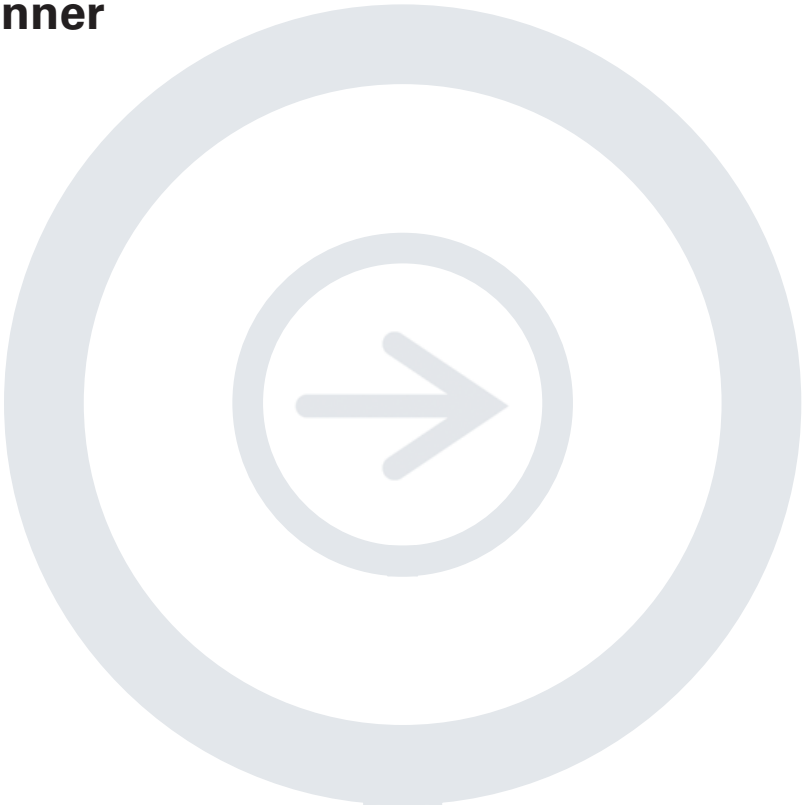
Chemiluminescent Western Blots

Frequently Asked Questions

Developed for:

Odyssey® Fc Imaging System

and C-DiGit® Blot Scanner



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November 2013. The most recent version of this protocol is posted at http://biosupport.licor.com

1. Blocking Buffer

1.1 Can I dilute the HRP-conjugated secondary antibodies in the Odyssey Blocking Buffer?

No. Odyssey Blocking Buffer contains sodium azide as a preservative. Sodium azide binds irreversibly to the HRP enzyme, inhibiting the binding of the substrate and slowing the chemiluminescent reaction. This results in less light production that may affect the appearance of less intense bands, or even the entire blot. For optimal results, do not use any solutions containing sodium azide for chemiluminescent Western blotting.

1.2 Can I use the Odyssey Blocking Buffer to block my blot?

Yes. Use only for the blocking step and be aware that the sodium azide from the Odyssey Blocking Buffer may still be present on the membrane at the detection step and will bind to the HRP enzyme, resulting in reduced light production and less intense bands.

1.3 Can I use milk-based blockers?

Yes. Milk-based blockers can be used for chemiluminescent detection but should be avoided when detecting phosphoproteins or glycoproteins. Milk-based blockers may contain endogenous biotin and glycoproteins, resulting in higher background on the membrane.

1.4 What is the best blocker for chemiluminescent Western blots?

It is best to try several blockers to find the one that gives the most satisfying data for each antigen and antibody pair. There is no one best blocker for all conditions.

2. Primary and Secondary Antibodies

2.1 Why is the signal missing in the middle of the bands?

Too much secondary antibody on the membrane results in consumption of all the substrate in that area. Without substrate, there is no chemiluminescent signal and a blank spot appears in the center of the band. Try different dilutions of the primary and secondary antibodies to find which gives best results, or try changing the substrate.

2.2 Does it matter where I purchased the HRP-conjugated secondary antibodies?

The reactivity of secondary antibodies ranges widely between vendors. The ratio of HRP to antibody varies as well, and may affect the detection of the target. LI-COR offers high-quality secondary antibodies for chemiluminescent Western blotting. WesternSure™ Goat anti-Mouse HRP (P/N 926-80010), and WesternSure Goat anti-Rabbit HRP (P/N 926-80011) are optimized for use with WesternSure PREMIUM Chemiluminescent Substrate (926-95000 and 926-95010) and are recommended for digital imaging.

2.3 Should the HRP-conjugated secondary antibodies be highly cross-adsorbed?

Highly cross-adsorbed secondary antibodies are essential for two-channel, multiplex detection; however, they are not necessary for detection of a single target.

3. Washing Buffer

3.1 Does it matter how I wash the membranes after antibody incubation?

Yes. Adequately washing membranes will greatly improve the appearance of the chemiluminescent Western blot. Wash membranes with a saline-buffered solution containing 0.05 to 0.1% of a non-ionic detergent such as Tween® 20. Wash 4 times for 5 minutes each time, with ample wash solution, on a shaker or rotator.

4. Substrate

4.1 Which substrate do I use?

There are a wide variety of chemiluminescent substrates for HRP detection. Substrates that provide longer duration and mid-femtogram levels of sensitivity are recommended for best performance and sensitivity on digital imaging systems. WesternSure PREMIUM chemiluminescent substrate (LI-COR P/N 926-95000 and 926-95010) provides higher reactivity and longer duration and has been optimized for use with Odyssey Fc Imaging System and C-DiGit Blot Scanner.

4.2 How do I apply the substrate?

Make sure the substrate is at room temperature before use. Substrate should be applied by following the manufacturer's suggested methods for the amount of substrate to use and the incubation time prior to imaging. The substrate can be added directly to the sample side of the membrane (either by pipetting the substrate on the sample surface, or by pre-incubating the membrane in the substrate), allowed to incubate, and imaged without having to wrap the membrane before scanning. When choosing this method, the addition of a sheet protector over the top of the blot can prevent possible drying.

4.3 The membrane dried during imaging. Can I apply more substrate and image again?

No. Applying more substrate to a dried blot will likely result in high background.

4.4 How do I keep the membrane from drying out?

For Odyssey Fc, place a clear, flat plastic covering on the chemiluminescent Western blot to keep the substrate in contact with the HRP enzyme and to prevent the blot from drying out. When using the C-DiGit Blot Scanner, the membrane should not dry out in the amount of time it takes to complete a scan; however, to maintain moisture on the membrane for an extended amount of time, place a clear, flat plastic covering on top of the membrane to keep the substrate in contact with the HRP enzyme and to prevent the blot from drying out. Membranes can also be placed in a clear, flat plastic covering prior to scanning. Make sure there is no plastic extending beyond the scanning surface and into the outer lid seal.

5. Imaging

5.1 Can I use the Odyssey Fc Imaging Tray multiple times?

It is important to image with a clean tray to prevent unwanted background, so you may want to use a new tray. You can clean a previously-used tray with ultrapure water or methanol to remove any traces of substrate or dye. If you have cleaned a used tray, image the tray by itself first to see if there is any contamination left. If there is still signal detected, clean the tray again with ultrapure water or methanol and re-image. If necessary, dispose of the contaminated tray and use a new tray.

5.2 Can I adjust the brightness and contrast of an image to enhance sensitivity?

Yes. All image adjustment methods using Image Studio Software will not alter the data collected, or the quantification of the data. For detailed instructions on how to make these adjustments, visit *www.licor.com/ISSupport*.

Related Resources

- How to Maximize Sensitivity on Chemiluminescent Western Blots
  - *Video:* (www.licor.com/ChemiVideo)
  - *Technical Note:* (www.licor.com/ChemiTech)

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