

Product Number

926-80020, 20 mL

926-80100, 100 mL

926-80200, 200 mL

926-80500, 500 mL

Contents: WesternSure™
ECL Luminol Enhancer
Solution; WesternSure ECL
Stable Peroxide Solution

Storage: Room Temperature

September 2014

Updates available at:

<http://www.licor.com/packinserts>

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LI-COR

4647 Superior Street
Lincoln, Nebraska 68504 USA
Biotechnology Support U.S.: 800-645-4260
International: 402-467-0700
FAX: 402-467-0819

LI-COR GmbH Germany, Serving Europe,
Middle East, and Africa: +49 (0) 6172 17 17 771
LI-COR UK Ltd., Serving UK, Denmark, Finland,
Ireland, Norway, and Sweden: +44 (0) 1223 422104
All other countries, contact LI-COR Biosciences
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WesternSure™ ECL Substrate

Introduction

WesternSure ECL Substrate is a highly-sensitive, enhanced substrate for detecting horseradish peroxidase (HRP) on immunoblots. The Substrate is optimized for use with the LI-COR C-DiGit® Blot Scanner and the Odyssey® Fc Imaging System. WesternSure ECL Substrate is designed for high sensitivity, making it ideal for use with imaging systems or film.

Protocol

CAUTION: For optimum sensitivity, the blot should be scanned immediately after the five-minute incubation with Substrate. Any delay may affect sensitivity.

NOTE: Optimize the antigen and antibody concentrations as well as blocking conditions for each experimental system. WesternSure HRP-conjugated secondary antibodies (LI-COR P/N 926-80010 or 926-80011) are designed for use with this substrate. HRP-conjugated secondary antibodies from other vendors may be suitable; optimization may be required. Avoid buffers containing sodium azide, as it will inhibit HRP activity.

1. Dilute primary antibody as recommended by the vendor.
2. Dilute WesternSure HRP Secondary Antibody 1:5,000 - 1:100,000 (LI-COR P/N 926-80010 or 926-80011).
3. Prepare Working Solution by mixing equal parts of the Luminol Enhancer Solution and the Stable Peroxide Solution. Use 0.1 mL Working Solution per cm² of membrane (e.g. ~5 - 6 mL for a mini blot).
NOTE: Prepare working solution at time of use; do not store working solution.
4. Incubate blot 5 minutes in substrate Working Solution.
5. Drain excess reagent and immediately expose to film, or capture digital image on the C-DiGit Blot Scanner or Odyssey Fc Imaging System.
6. For imaging on the C-DiGit Blot Scanner, place the wet blot directly on the scanning surface, protein side facing down. Place a plastic sheet over the membrane to prevent drying. Select 'Standard' or 'High' sensitivity setting, then click 'Start'.
7. For imaging on the Odyssey Fc Imaging System, place the wet blot in a clear plastic sleeve to prevent drying. Place the blot in the tray, protein side facing up. Select the acquisition time, then click 'Start'.
8. For film, cover the blot with clear plastic wrap or a clear plastic sleeve. Place the blot in a film cassette with the protein side facing up. Place film on top of blot and expose for the desired length of time.

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