

Product Number

926-32236

Quantity: 0.5 mg

Storage: 4 °C

Revised: November 2010

Updates available at:

<http://biosupport.licor.com>

**Limitation of Liability and
Limited Use Label License**

LI-COR IRDye® Infrared Dyes and reagent products are offered for research purposes only and are not intended for human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the amount of product purchased and the components of the product in research conducted by the buyer (whether the buyer is a not-for-profit, academic or for-profit entity). The buyer shall not sell or otherwise transfer this product, its components, or materials made there from to any third party. Buyer shall not use this product or its components for commercial purposes. The term "commercial purposes" shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components (i) in manufacturing, (ii) to provide a service, information or data, (iii) for therapeutic, diagnostic or prophylactic purposes, or (iv) for resale, whether or not such product or its components are resold for use in research. The use of this product by the buyer constitutes agreement with the terms of this limited use label license for LI-COR IRDye infrared dyes and reagent products. Inquiries regarding the licensing of one or more IRDye infrared dyes should be submitted by e-mail to busdev@licor.com.

LI-COR BIOSCIENCES DOES NOT PROVIDE RESEARCH ADVICE OR DETERMINE OR RECOMMEND ANY POTENTIAL USES FOR IRDYE INFRARED DYES AND REAGENT PRODUCTS. LI-COR BIOSCIENCES MAKES NO WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED, AS TO ANY MATTER INCLUDING, BUT NOT LIMITED TO, WARRANTY OF FITNESS FOR PURPOSE, OR MERCHANTABILITY OR RESULTS OBTAINED FROM USE OF IRDYE INFRARED DYES. IN NO EVENT SHALL LI-COR BE LIABLE FOR LOST PROFITS, CONSEQUENTIAL, EXEMPLARY, SPECIAL, DIRECT, INCIDENTAL, OR PUNITIVE DAMAGES, OR ATTORNEY FEES, EVEN IF LI-COR HAD BEEN ADVISED OF, KNEW, OR SHOULD HAVE KNOWN, OF THE POSSIBILITIES THEREOF. NO EMPLOYEE, AGENT OR REPRESENTATIVE OF LI-COR HAS THE AUTHORITY TO BIND LI-COR TO ANY ORAL REPRESENTATION OR WARRANTY EXCEPT AS SPECIFICALLY SET FORTH HEREIN.

© 2009 LI-COR, Inc. LI-COR is an ISO 9001 registered company. IRDye infrared dyes are covered by U.S. and foreign patents and patents pending. LI-COR, Chemi-IR, IRDye, and Odyssey are trademarks or registered trademarks of LI-COR, Inc. in the U.S. and other countries. All other trademarks belong to their respective owners. For patent information, visit www.licor.com/patents.

Doc #988-10291

LI-COR®

4647 Superior Street • P.O. Box 4000
Lincoln, Nebraska 68504 USA
North America: 800-645-4267
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. UK, Serving UK, Ireland, and Scandinavia: +44 (0) 1223 422104

www.licor.com

Chemi-IR™ Detection Kit, 100 Western Blots

Applications

The Chemi-IR Detection Kit provides a way to convert chemiluminescent Western blots to near-infrared fluorescence. Incubation of Western blots with IRDye® 800CW Rabbit anti-HRP enables detection of HRP-conjugated antibody.

For additional protocols, please reference the Odyssey® or Aeries Imaging System Applications Manuals included with all instrument installations, or download them from <http://biosupport.licor.com>.

Kit Components

- Chemi-IR Conjugate (IRDye 800CW Rabbit anti-HRP, 0.5 mg, lyophilized)
- Chemi-IR Diluent (500 mL)
- Western Incubation Box, Large (11.8 x 8.9 x 2.9 cm)

Additional Reagents Required

- Western blot detected with HRP conjugated antibody
- 1X PBST (0.1% Tween® 20)
- 1X PBS
- 20% Tween 20
- 20% SDS (If using PVDF membrane)

IRDye 800CW Rabbit anti-HRP Properties

Antibody Concentration: 1.0 mg/mL when reconstituted as directed

Fluorophore: IRDye 800CW

Excitation Wavelength: 778 nm (in PBS)

Emission Wavelength: 795 nm (in PBS)

Fluorophore/Protein Ratio: moles IRDye 800CW: 1 mole IgG

Form of Antibody: IRDye 800CW conjugated purified immunoglobulin, lyophilized in phosphate buffered saline, pH 7.4. Contains 10 mg/mL BSA (IgG and protease free) as stabilizer and 0.01% sodium azide as preservative.

Warning: Sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.

Immunogen: Peroxidase from horseradish roots

Purity and Specificity

The antibody was purified from antisera by immunoaffinity chromatography using antigens coupled to agarose beads. Based on immunoelectrophoresis and/or ELISA, the antibody reacts with peroxidase from horseradish roots. It may cross-react with peroxidase from other sources. The conjugate has been specifically tested and qualified for Western blot applications.

Reconstitution and Storage

Protect from light. Store at 4°C prior to reconstitution. Reconstitute contents of vial with 0.5 mL sterile distilled water. Mix gently by inverting, and allow to rehydrate for at least 30 minutes before use. Centrifuge product if solution is not completely clear after standing at room temperature. Dilute immediately prior to use only. This product is stable for up to 3 months at 4°C when stored undiluted and protected from light as directed.

Diluent Preparation

Add 5.0 mL of 20% Tween 20 to 500 mL bottle of Chemi-IR Diluent. Mix well by inversion. This will be a final concentration of approximately 0.2% Tween 20.

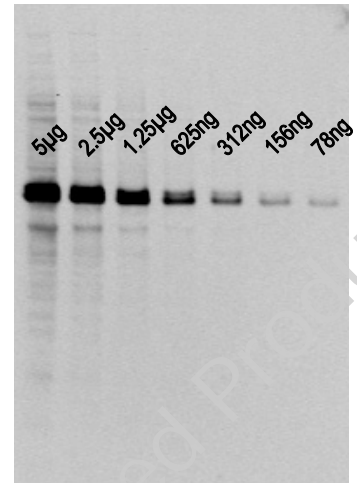
Western Detection

A Western blot that has been incubated with an HRP conjugated antibody can be detected with IRDye 800CW anti-HRP. Optimal results are obtained when using a Western blot that has not been processed with a chemiluminescent substrate.

Notes

- Ink from most pens and markers will fluoresce on Odyssey. The ink may wash off and re-deposit elsewhere on the membrane, creating blotches and streaks. Mark with pencil or the provided Odyssey pen to avoid this problem. Use pencil for PVDF membrane (wetting in methanol will cause ink to run).
 - Odyssey Blocking Buffer contains 0.1% sodium azide; do not use as HRP-conjugated antibody diluents.
1. Dilute IRDye 800CW Rabbit anti-HRP 1:1000 (final concentration of 1 $\mu\text{g}/\text{mL}$) in Chemi-IR Diluent. If using large Western Incubation Box included with the kit, dilute 10 μL of IRDye 800CW Rabbit anti-HRP into 10 mL of Chemi-IR Diluent.
Reminder: Add Tween 20 to the Chemi-IR Diluent before use.
Important: If using PVDF membrane, add SDS to a final concentration of 0.002 to 0.01% to the Chemi-IR Diluent.
 2. Add diluted IRDye 800CW Rabbit anti-HRP conjugate to blot and incubate for 1 hour at room temperature on platform shaker, protected from light.
 3. Decant diluted conjugate and rinse blot briefly in 15 mL of 1X PBST. Decant. Add 15 mL of 1X PBST and incubate at room temperature on platform shaker protected from light for 5 minutes. Decant. Repeat wash step 2 more times.
 4. Rinse with 15 mL of 1X PBS.
 5. Scan wet on the Odyssey Infrared Imaging System using the *Membrane* preset with the 800 nm Channel. Start with Scan Intensity set at 6 and adjust as needed.

For *in vitro* research use only; not for diagnostic or therapeutic use. This product is not a medical device.



Serial dilutions of A431 lysate were run by SDS PAGE and transferred to nitrocellulose. The blot was probed with monoclonal anti-p53 followed by Donkey anti-Mouse HRP. The blot was detected on the Odyssey Infrared Imaging System with the Chemi-IR Detection Kit (IRDye 800CW Rabbit Anti-HRP).