Product Number
926-32236

Quantity: 0.5 mg

Storage: 4 °C

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Updates available at:
http://biosupport.licor.com

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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 Aerius 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 µg/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.

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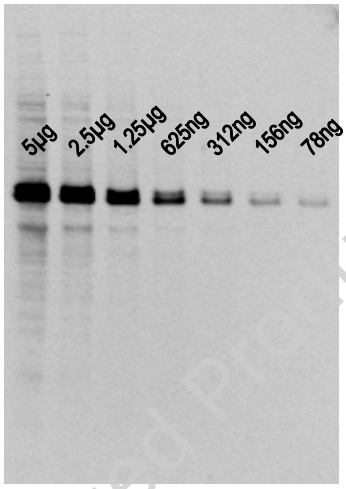
For *in vitro* research use only; not for diagnostic or therapeutic use. This product is not a medical device.



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).