

**ChemiLucent™**  
**WESTERN BLOT DETECTION SYSTEM**

**CATALOG NUMBER:** 2600

**LOT NUMBER:** xxxxx

**QUANTITY:** 1 Kit (sufficient for 5,000 cm<sup>2</sup> membrane)

**DESCRIPTION:** The Chemicon *ChemiLucent*<sup>™</sup> is based on a luminol-HRP-chemiluminescence reaction. The chemiluminescence light emission can be recorded by a short exposure to blue-light sensitive autoradiography films. *ChemiLucent*<sup>™</sup> will allow detection of sub-picogram quantity of antigens with lower background.

**KIT COMPONENTS:**

Luminol/Enhancer Solution (90069)	250 mL
Peroxide Solution (90090)	1.0 mL
Peroxide Buffer (90089)	250 mL

**ITEMS NOT SUPPLIED:**

1. Primary antibodies
2. Secondary antibodies-HRP-conjugates (Available from Chemicon. See Related Products for more information.)
3. TBST & PBST Buffers
4. Tween-20
5. BLOT-QuickBlocker

**PROTOCOL:**

For conserving reagent solutions, the protocol should be carried out in a container that is just large enough to accommodate the transfer membrane. Preferably, the primary and secondary antibody treatments, and the reaction with the detection reagents should be carried out in sealable bags where the reaction volume is minimized.

**1. Blocking**

After electrophoretic transfer of protein to an appropriate membrane, block membrane with 5% solution of BLOT-*QuickBlocker*<sup>™</sup> in TBST or PBST buffer, containing 0.1% Tween-20. Note: BLOT-*QuickBlocker*<sup>™</sup> contains modified milk protein.

Incubate the membrane in an appropriate volume of 5% BLOT-*QuickBlocker* solution at room temperature for 20-30 minutes. Some experiments may require a longer incubation in the blocking step. Agitate the membrane on an orbital shaker during the blocking step.

**2. Wash**

After blocking is complete, wash the membrane three times in PBST or TBST buffer containing 0.1% Tween-20. Use a large volume of washing buffer in a tray for the washing step. Each wash cycle involves agitating the membrane 5 minutes in the washing buffer.

**3. Primary Antibody Treatment**

After washing step, transfer the washed membrane to an appropriate primary antibody. Use a sealable bag for conserving primary antibody. Make an appropriate dilution of primary antibody in 5-10 mL of 1XTBST buffer containing 0.5% BLOT-*QuickBlocker* and incubate the washed membrane for 90 minutes at room temperature or overnight at 2-8°C.

**NOTE:** Optimal dilution of primary antibody must be determined by a separate dot blot experiment, prior to running the *ChemiLucent*<sup>™</sup> protocol.

4. Wash the membrane 5 times in washing buffer, as described in step 2.

5. Make appropriate dilution of secondary antibody-HRP-conjugate in 5-10 mL of 1XTBST buffer. Use a sealable bag for conserving secondary antibody. Incubate the membrane in the secondary antibody-HRP-conjugate prepared in 1XTBST buffer at room temperature for 60-90 minutes.

**NOTE:** Optimal dilution of secondary antibody must be determined separately, either by a dot blot experiment or following the manufacturer's instructions.

6. Wash the membrane with 1XTBST buffer at room temperature 4 times.

Transfer the membrane into a large tray for washing. For each wash cycle use 50-100 mL 1XTBST buffer. Agitate the membrane on an orbital shaker for 10-15 minutes during each wash cycle.

#### 7. Prepare *ChemiLucent*<sup>™</sup> Working Solution

Prepare a 1:1,000 dilution of Peroxide Solution with Peroxide Buffer. Mix equal parts of *ChemiLucent*<sup>™</sup> luminol/enhancer solution and peroxide solution. This working solution is stable for several hours at room temperature.

#### 8. Develop the Membrane

After wash cycles, transfer the membrane into the *ChemiLucent*<sup>™</sup> working solution. Use a sealable bag for conserving reagents. Incubate the membrane in the *ChemiLucent*<sup>™</sup> working solution for 3 minutes at room temperature. Drain the detection reagent from the reaction bag and expose the membrane to the film. Do not wash the membrane.

#### 9. Expose to Film

Place the membrane (in reaction bag), protein side up in the film cassette. Place a film on the top of the membrane and expose the film, initially 10-20 seconds and then re-exposed for the optimal time as needed.

#### 10. Re-blot

The blot can be reprobed if necessary.

#### TROUBLESHOOTING:

##### 1. No Signal

- Protein is not transferred completely.
- Protein is over transferred and passed through the membrane.
- Primary antibody is too dilute.
- Change the dilution of the secondary antibody-peroxidase conjugate.
- Use fresh detection reagent and detection buffer.

##### 2. High Background

- Incomplete blocking, use fresh blocking buffer.
- There is too much antigen per lane of SDS-PAGE.
- Reduce the concentration of the primary antibody.

#### STORAGE:

Store kit components at 2° to 8°C for up to one year from date of purchase. **Note:** Do not store diluted Peroxide Solution or *ChemiLucent* Working Solution. These solutions are only stable for several hours after mixing.

**Related Chemicon Products:**

<b>Product Description</b>	<b>Qty</b>	<b>Cat. No.</b>
<b>BLOT-QuickBlocker™</b> A novel blocking protein which produces a semi-clear solution. This allows users the option to examine their blot membranes during the blocking procedure	200 gm	2080
Goat anti-Mouse IgG (H&L)-HRP	2 mL	AP124P
Goat anti-Rabbit IgG (H&L)-HRP	2 mL	AP132P
Goat anti-Rat IgG (H&L)-HRP	2 mL	AP136P
Goat anti-Human IgG (H&L)-HRP	2 mL	AP112P
Goat anti-Goat IgG (H&L)-HRP	1.5 mL	AP106P
<b>BLOT-FastStain™</b> For reversible staining of protein on transfer membranes. Stains only protein and leaves background absolutely untouched and brilliant white leading to exceptional band visibility. Detects as little as 0.3 ng BSA	25 Blots/Kits	2076

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