Lumigen® ECL Ultra (TMA-6)

Chemiluminescent Reagent

Product Overview

Catalog Number	**TMA-100**
Contents	Solution A - 50 mL Solution B - 50 mL For working solution, mix solutions A and B in the ratio of 1:1
Description	**Lumigen ECL Ultra** is recommended primarily for ultra-sensitive detection of Western blotted proteins that are bound with antibodies conjugated with horseradish peroxidase (HRP).

Note: Lumigen ECL Ultra is invented, developed and manufactured by Lumigen

Product Characteristics and Applications

- Allows convenient imaging over several hours.
- Short exposures (15 to 30 sec.); high signal intensity permits rapid visualization of chemiluminescent bands proteins on Western blots.

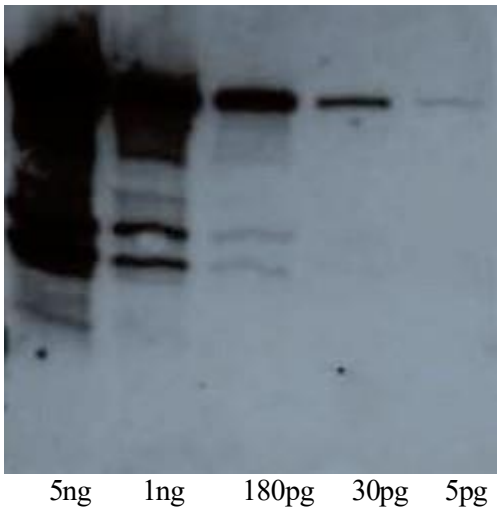

Figure 1: Western blot of ß-galactosidase protein detected with Lumigen ECL Ultra

Suggested Product Handling Instructions

Storage:

Store bottled product at 2 - 8ºC.
Protect from exposure to direct light.
Do not freeze.

Use:

***Use in subdued light. Indirect incandescent lighting is preferred. Exposure to direct light will cause elevated background.**

1) Allow solutions A and B to equilibrate to room temperature (Approximately 1 hour for 100 mL)
2) Gently invert (4-5 times) solutions A and B in their packaged containers to assure homogeneity prior to dispensing. **Avoid vigorous agitation of reagent.**
3) Dispense the amount needed of solutions A and B into separate containers. Containers should be opaque or covered with aluminum foil to protect from direct light (daylight or artificial).
4) For working solution, mix solutions A and B in a ratio of 1:1 in a new container.
5) In subdued light, add the working solution onto membrane with HRP bound protein or nucleic acids, and let stand for 2 minutes.
6) Place the membrane sandwiched between two clear plastic sheets. Wipe away excess solution with a tissue leaving a thin layer of solution on the membrane.
7) Capture chemiluminescent signals on an x-ray film or using an imager.

Repackaging:

Repackaging of Lumigen ECL Ultra is discouraged as reliability can be compromised by contamination. Bottling is available from Lumigen to suit the volume demands for your specific work process.

If you choose to repackage, new opaque HDPE or PP plastic containers are required. **Reusing and washing containers can lead to contamination and subsequent high background.**

Western Blotting - Equipment and Materials Required

- Vertical slab gel equipment.
- Acrylamide gels, freshly cast or precast.
- Power supply for electrophoresis.
- Nitrocellulose or PVDF membranes.
- Methanol (for PVDF membrane)
- Protein transfer equipment.
- Transfer buffer (Tris/Glycine/Methanol).
- Buffers such as Tris buffered saline (TBS) or phosphate buffered saline (PBS).
- Wash buffers (TBS or PBS) containing 0.05% to 0.1% Tween -20.
- Blocking reagent such as 5% non-fat dry milk in wash buffer or any other commercially available blocking reagents.
- Primary antibody (target specific), and HRP labeled secondary antibody.
- Platform shaker for antibody incubation and washing the membrane.
- X-ray film.
- X-ray film processing equipment with developing and fixing reagents.

Important Notes and Precautions

Any time a new chemiluminescent reagent is used for the detection of proteins on Western blots, it is essential that the concentrations of target specific primary antibody and the HRP labeled secondary antibody are titrated first to determine the optimal dilution combination of these antibodies. The antibody stocks from commercial vendors vary in their binding specificity and protein concentration. As a general rule, 1mg/mL antibody stocks may be diluted and used in the range of 1 to 0.1 µg/mL (1:1000 to 1:10,000) for primary antibody and 0.1 to 0.01 µg/mL (1:10,000 to 1:100,000) for secondary antibody. Other variables that influence the detection sensitivity of the chemiluminescent reagent are the type of membrane used for blotting, the efficiency of protein transfer on to the membrane, and the blocking agent used to minimize non- specific background. Maintaining temperature during antibody incubation may help to achieve consistent results. Always make sure the membranes are completely covered with antibody and washing buffers and not let the membranes dry at any stage prior to chemiluminescent detection. To reduce cross-contamination, use fresh plastic ware for incubating the membranes with primary and secondary antibodies, and when treating the membrane with the chemiluminescent reagent. Wear gloves when handling membrane blots and X-ray films.

Western Blotting Procedure and Chemiluminescent Detection Steps:

1. Electrophorese the proteins of interest on a polyacrylamide gel. Follow the standard protocols to prepare protein samples including measurement of protein concentration, adding the loading buffer, and loading an appropriate amount of sample to the wells. For the gel, choose a concentration of acrylamide based on the size of proteins to be resolved.

2. Transfer the proteins from the gel onto a nitrocellulose or PVDF membrane using standard gel transfer apparatus and buffers. Before the transfer, prepare the membranes following protocols appropriate to the type of membrane such as immersing PVDF membrane first in methanol and then equilibrating in transfer buffer.

3. Remove the membrane blot from the transfer apparatus and immediately immerse in blocking reagent such as 5% non-fat dry milk prepared in wash buffer or any commercially available blocking reagent. Blocking can be done for 1 hour at ambient temperature (22-25°C) with moderate shaking on a platform or overnight at 2-8°C without shaking.

4. Remove the blocking reagent and incubate the membrane with target specific primary antibody diluted in blocking reagent for 1 hour with gentle shaking.

5. Remove the primary antibody and rinse the membrane 2-3 times with fresh wash buffer followed by 2-3 longer washes (about 10 minutes) with moderate shaking. Use generous amounts of wash buffer to keep the membranes completely immersed while washing.

6. Remove the wash buffer and incubate the membrane with HRP conjugated secondary antibody diluted in blocking reagent for 1 hour with gentle shaking.

7. Remove the secondary antibody and rinse the membrane 2-3 times with fresh wash buffer followed by 3-4 longer washes (about 10 minutes) with moderate shaking. Use generous amounts of wash buffer to keep the membranes completely immersed while washing.

Note the following steps 8, 9 and 10 are performed under subdued light conditions.

8. While washing the membrane in step 7 above, prepare the working solution of Lumigen ECL Ultra substrate by mixing solutions A and B in the ratio of 1:1.

9. Place the membrane from step 7 in a fresh plastic dish and add the working solution of Lumigen ECL Ultra onto the membrane. Let the membrane stand for 2-3 minutes in the substrate.

10. For chemiluminescent detection, remove the membrane from the plastic dish and place it sandwiched between two clear plastic sheets. Wipe away excess solution with an absorbent tissue leaving a thin layer of solution on the membrane without any air

bubbles. Seal the edges of plastic sheets with a sticky tape.

11. Turn off the subdued lights and turn on the red safe light. Expose the protein side of the membrane to an X-ray film in a film cassette or sandwiched between two glass plates.

Troubleshooting Tips

There are mainly two types of problems associated with the detection of proteins on Western blots.

1. **Signal problems:** Weak or distorted signal bands
2. **Background problems:** High non-specific background and spots

Signal Problems	Possible Causes	Troubleshooting Tips
1. Hollow protein bands on the X- ray film	High amount of HRP antibody depleted the substrate at the band	Use more diluted HRP antibody
2. Brown or yellow protein bands on the membrane	High amount of HRP antibody	Use more diluted HRP antibody
3. Protein signal bands fade away quickly	High amount of HRP antibody with low amount of protein	Use more diluted HRP antibody and/or increase amount of protein loaded
4. Weak protein signal bands	• Low amount of protein loaded • Low amount of antibodies used • Inefficient protein transfer	• Load higher amounts of protein into wells • Use higher amount of antibodies • Check protein transfer apparatus, buffers and power supply
5. No signal	• Protein not transferred to membrane • Antibodies did not bind to the target protein band • Inactive HRP enzyme • Expired substrate	• Check protein transfer components • Replace antibodies, both primary and secondary with newer lots • Replace substrate with newer lot
6. Hollow spots in protein bands	• Inefficient protein transfer due to air bubbles between the gel and membrane • Insufficient/uneven wetting of membrane • Air bubbles on the protein bands when exposed to X-ray film	• Make sure to remove air bubbles while making the gel-membrane sandwich • Wet the membrane in adequate amount of water for at least 5 minutes • Prior to X-ray film exposure wipe away air bubbles on the membrane

Background Problems	Possible Causes	Troubleshooting Tips
1. Dark membrane background on the X-ray film	• Use of high amount of HRP antibody • Insufficient washing of the membrane • Inadequate blocking duration • Inappropriate blocking reagent • Membrane dried during any of the steps • Long X-ray film exposure time	• Use more diluted HRP antibody • Increase wash buffer volume, wash duration, number of washes, and shaking intensity • Block longer period of time • Switch to another blocking reagent • Keep the membrane wet until exposed to X-ray film • Take shorter exposures
2. Membrane visibly glows in the dark room	• Use of high amount of HRP antibody • Inappropriate blocking reagent • Membrane dried during any of the steps • Inappropriate membrane	• Use more diluted HRP antibody • Switch to another blocking reagent • Keep the membrane wet until exposed to X-ray film • Switch to a different membrane
3. Dark spots on the membrane X-ray exposure	• Use of HRP antibody that aggregated during storage • Gel or membrane over heated during electrophoresis and/or transfer	• Spin down the antibody aggregates in a microcentrifuge for a few seconds and use the supernatant antibody for dilution • Lower voltage during electrophoresis and/or keep the transfer apparatus cool with frozen ice