（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Excellent Chemiluminescent Substrate Kit

Catalog No: AGEL3539
50 mL

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Description

Excellent Chemiluminescent Substrate is an enhanced chemiluminescent (ECL) substrate for horseradish peroxidase (HRP) enzyme that provides low picogram detection of proteins in Western blot and ELISA applications.

Excellent Chemiluminescent Substrate emits light for longer and chemiluminescent signal at more than twice the intensity of other entry-level luminol-based horseradish peroxidase detection systems while also maintaining sensitivity and low background. In addition, the range of the calibration curve is much wider than other competitors. Also, the greater signal intensity is achieved using less primary and secondary antibody than typical ECL substrates. All of this for about 30% less cost than comparable products.

Product features

- **ECL substrate** – An enhanced chemiluminescent substrate for detection of horseradish peroxidase (HRP) activity of antibodies and other ELISA or Western blot probes.
- **Economy** – Costs less per milliliter than other similarly sensitive chemiluminescent substrates.
- **Picogram sensitivity** – Highly sensitive for the rapid development of a wide range of protein levels.
- **Stable light output** – Long-duration signal makes it possible to make multiple exposures to film.
- **High intensity** – Signal is twice as strong as output from other luminol-based systems.
- **Excellent stability** – 8-hour working solution stability; 1-year kit stability at 2~8℃.
- **Saves antibody** – Requires much less (more dilute) primary and secondary antibodies per blot.
- **Wide range of the calibration curve** –Calibration curves range from 4.00–8000 pg, R2 is 0.992.

Kit components

Item	50 mL	100 mL	250 mL	500 mL	1000 mL	Storage
Substrate A	25mL	50 mL	125 mL	250 mL	500 mL	2~8℃
Substrate B	25 mL	50 mL	125 mL	250 mL	500 mL	
Product Descrition	1 copy					

Detailed Western Blotting Procedure

1. Remove the blot from the transfer apparatus and block nonspecific sites with **Blocking Reagent** for 20-60 minutes at room temperature (RT) with shaking. For best results, block for 1 hour at RT.

   Note: Avoid using milk as a blocking reagent when using Avidin/Biotin systems because milk contains variable amounts of endogenous biotin.

2. Remove the **Blocking Reagent** and add the appropriate primary antibody dilution. Incubate the blot for 1 hour with shaking. If desired, blots may be incubated with primary antibody overnight at 2-8°C.
3. Wash the membrane by suspending it in **Wash Buffer** and agitating for ≥ 5 minutes. Replace Wash Buffer at least 4-6 times. Increasing the wash buffer volume and/or the number of washes may help reducing background.

   Note: Briefly rinsing membrane in wash buffer before incubation will increase wash efficiency.

4. Incubate the blot with the appropriate HRP-conjugate dilution for 1 hour at RT with shaking.
5. Repeat **Step 3** to remove non-bound HRP-conjugate.

   Note: Membrane must be thoroughly washed after incubation with the HRP-conjugate.

6. Prepare Working Solution by mixing equal parts of the **Substrate A** and **Substrate B**. Use 0.1 mL Working Solution per cm^2 of membrane. The **Working Solution** is stable for 8 hours at RT.

   Note: Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Typical laboratory lighting will not harm the Working Solution.

7. Incubate the blot with **Working Solution** for 5 minutes.
8. Remove the blot from **Working Solution** and place it in a plastic membrane protector, a plastic sheet protector or plastic wrap may be used. Use an absorbent apparatus to remove excess liquid and to carefully press out any bubbles between the blot and surface of the membrane protector.
9. Place the protected membrane in a film cassette with the protein side facing up. Turn off all lights except those appropriate for film exposure (e.g., a red safelight).

   Note: Film must remain dry during exposure. For optimal results, perform the following precautions:
   - Make sure excess substrate is removed from the membrane and the membrane protector.
   - Use gloves during the entire film-handling process.
   - Never place a blot on developed film, as there may be chemicals on the film that will reduce signal.

10. Carefully place a piece of film on top of the membrane. A recommended first exposure time is 60 seconds. Exposure time may be varied to achieve optimal results. Enhanced or pre-flashed film is unnecessary.

> **Note:** The exposure time may be varied to achieve optimal results. If the signal is too intense, reduce exposure time or optimize the system by decreasing the antigen and/or antibody concentrations.
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> Light emission is most intense during the first 5-30 minutes after substrate incubation. Light emission will continue for several hours, but will decrease with time. Longer exposure times may be necessary as the blot ages.
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> **Attention:** Light emission is intense and any movement between the film and membrane may cause artifacts on the film.