

ECL Enhanced Femto Kit (1fg)

Cat. No.: BRK0094

Size: 100 mL / 500 mL

◆ Description

ECL Enhanced Femto Kit (1fg) , is a high-sensitivity chemiluminescent (ECL) HRP substrate that enables users to achieve lower femtogram-level protein detection ($\sim 1 \times 10^{-14}$ g) during Western blot analysis, with significant antibody conservation. It delivers bright signals and lower picogram detection sensitivity for Western blot experiments utilizing horseradish peroxidase (HRP) conjugates. Compatible with a wide range of membranes, blocking buffers, and antibody dilutions, this ECL substrate meets diverse Western blotting application requirements through its exceptional performance, versatility, and cost-effectiveness.

◆ Features

- 1.High Sensitivity: Detects lower picogram-level protein bands on nitrocellulose membranes or PVDF membranes.
 - 2.Long Signal Duration: Under optimized conditions, the blot bands incubated with the substrate can continuously emit detectable light signals for 6–8 hours.
 - 3.Cost-Effective: Optimized formulation suitable for antibody detection at extremely low concentrations.
- Recommended dilutions:

Primary antibody: 1:1,000–1:5,000 (starting from a 1 mg/mL stock solution) or 0.2–1 μ g/mL;

Secondary antibody: 1:20,000–1:100,000 (starting from a 1 mg/mL stock solution) or 10–50 ng/mL.

◆ Product Components

Composition	Size 1	Size 2
SolutionI	50 mL	250 mL
SolutionII	50 mL	250 mL

◆ Storage

Store at 2–8°C in the dark for 24 months.

◆ Protocol (for reference only)

- 1.Remove the blot membrane, add an appropriate blocking buffer, and incubate with shaking at room temperature for 20–60 minutes to block non-specific protein binding sites on the membrane.
- 2.Take the membrane out of the blocking buffer and incubate it with the primary antibody working solution at room temperature for 1 hour with shaking; or incubate overnight at 2–8°C without shaking.
- 3.Add sufficient wash buffer to the membrane to ensure full coverage. Incubate with shaking for ≥ 5 minutes,

replace the wash buffer, and repeat this step 4–6 times. Increasing the wash buffer volume, number of washes, and washing time helps reduce background signals.

Note: ① A brief rinse of the membrane in wash buffer before incubation improves washing efficiency. ② It is critical to follow the recommended HRP-conjugated secondary antibody dilution as stated above.

4. Incubate the membrane with the HRP-conjugated secondary antibody working solution at room temperature for 1 hour with shaking.

5. Repeat Step 3 to remove unbound HRP-conjugated secondary antibody.

Note: Thorough washing is required after incubating the membrane with the HRP-conjugated secondary antibody.

6. Mix Solution I and Solution II at a 1:1 ratio to prepare the working solution. In this kit, Solution I is the substrate and should be stored in a light-resistant reagent bottle; Solution II is the oxidizer. The typical sampling order is to first take Solution I, change the pipette tip, and then take Solution II. Use 0.01–0.1 mL of working solution per cm² of membrane. The working solution is stable for 8 hours at room temperature.

Note: It is recommended to use the working solution immediately. Although it can still be used after being left at room temperature for several hours, its sensitivity will decrease slightly. Exposure to sunlight or other strong light may damage the working solution. For optimal results, store the working solution in an amber bottle or perform the operation in a dark room.

7. Incubate the blot membrane in the working solution for 5 minutes. Remove the membrane from the working solution and place it between a plastic sheet or clean plastic wrap. Blot excess liquid with absorbent paper, and carefully press out any air bubbles between the blot and the plastic wrap.

8. Place the membrane wrapped in plastic wrap into a film cassette with the protein side facing up. Turn off all lights except those suitable for film exposure (e.g., a red safety light).

Note: ① The film must remain dry during exposure; ② Ensure that excess substrate is completely removed from the membrane and plastic wrap; ③ Wear gloves throughout the film handling process.

9. Place an X-ray film over the membrane. An initial exposure time of 60 seconds is recommended. Adjust the exposure time thereafter to achieve optimal results. The chemiluminescent reaction is strongest within the first 5–30 minutes after substrate incubation. The reaction can last for several hours, but the signal intensity will decrease over time. If exposure is performed a long time after substrate incubation, a longer exposure time may be needed to obtain a strong signal. Longer exposure times may be required when using phosphorescence storage imaging systems (e.g., Bio-Rad Molecular Imager systems) or CCD cameras.

Note: Any movement between the film and the membrane may cause artificial non-specific signals on the film. Take full advantage of the 30-minute period with strong chemiluminescence for film exposure.

10. Develop the film using appropriate developer and fixer solutions. If the signal is too strong, shorten the

exposure time, or strip the blot membrane and re-detect with a lower antibody concentration.

◆ **Notice**

1. This product is for research use only and shall not be used in clinical diagnosis, treatment, or other related fields. Both Solution I and Solution II are harmful to humans; please take proper protective measures during operation. For your safety and health, wear a lab coat and disposable gloves when handling.
2. To achieve optimal results, all components of the system must be optimized, including sample load, primary and secondary antibody concentrations, as well as the types of membrane and blocking reagent.
3. Lower antibody concentrations are required for detection with this product compared to precipitating colorimetric HRP substrates. To optimize antibody concentrations, a systematic dot blot analysis is recommended.
4. No single blocking reagent is optimal for all systems; therefore, it is essential to identify the most suitable blocking buffer for each Western blot detection system. Blocking reagents may cross-react with antibodies, resulting in non-specific signals. Blocking buffers also affect the sensitivity of the system. Signal reduction or increased background may sometimes occur when switching from one substrate to another, which may be due to the incompatibility of the blocking buffer with the new detection system.
5. When using an avidin/biotin detection system, avoid using milk as a blocking reagent, as milk contains variable amounts of endogenous biotin, which can lead to high background signals.
6. Ensure sufficient volumes of wash buffer, blocking buffer, antibody solutions, and substrate working solution are used to guarantee that the blot membrane is completely covered by liquid throughout the experiment and prevent the membrane from drying out. Increasing the volumes of blocking buffer and wash buffer can reduce non-specific signals.
7. For optimal results, use an orbital shaker during the incubation steps.
8. Add Tween 20 (final concentration 0.05–0.1%) to the blocking buffer and diluted antibody solutions to reduce non-specific signals. Use high-quality products such as detergents stored in ampoules with low levels of peroxides and other impurities.
9. Do not use sodium azide as a preservative in buffers, as sodium azide is an inhibitor of HRP.
10. Avoid direct contact between hands and the membrane; wear gloves or use clean forceps during the experiment.
11. All equipment must be clean and free of contaminants. Metal instruments (e.g., scissors) must be free of visible rust, as rust may cause spot formation and high background.
12. The substrate working solution is stable for 8 hours at room temperature. Exposure to sunlight or any other strong light may damage the substrate. For optimal results, store the substrate working solution in an amber bottle and avoid prolonged exposure to any strong light. Brief exposure to standard laboratory lighting will not harm the working solution, which can be avoided by transferring to a darkroom for operation.
13. Recap all solutions tightly after use to prevent inactivation. This is especially important for Solution II, which

contains an oxidizer and is prone to inactivation by reduction.