ECL Enhanced Plus Kit

Cat.No: RM00021P

Size: 50mL（SolutionⅠ 50mL + SolutionⅡ 50mL）， 250mL（SolutionⅠ 250mL + SolutionⅡ 250mL）

◆ Product description

A luminol-based chemiluminescent kit for the detection of horseradish peroxidase (HRP) enzyme. The sensitivity can be reached at 1pg HRP. A new type of oxidant replaces unstable hydrogen peroxide, which improves the stability of the kit and can be stored for 1 year at room temperature. Once the working solution is catalyzed by HRP, it emits fluorescence at a specific wavelength (400-450nm), which can be exposed by X-ray film or CCD imaging system for Western Blot detection and chemiluminescence immunoassay.

◆ Product Features

1. Ultra-high sensitivity: detection of the low expression protein bands on nitrocellulose or PVDF membranes.
2. Longer signal duration: under some optimization, signal duration can be reached 6-8 hours.
3. Stability: Working solution stability within 24 hours
4. More affordable: under certain optimization, recommended primary antibody concentration is 1:1,000-1:5,000 dilution (0.2-1µg/mL); recommended secondary antibody concentration is 1:20,000-1:100,000 dilution (10–50 ng/mL)

◆ Product Components

Components	Size 1（50mL）	Size 2（250mL）
SolutionⅠ	50mL	250mL

Solution II	50mL	250mL

Storage Conditions

Store at 2-8°C, valid for 24 months.

Protocol

1. After the membrane transfer and blocking. Incubate membrane with 10ng-0.2μg/mL primary antibody for 1h or overnight. Wash the membrane and incubate with 10-50ng/mL secondary antibody for 30-60min .

2. Prepare the luminescence detection working solution by mixing equal parts of Solution I and Solution II (e.g., 1-2 mL per 10cm×10cm membrane)

Note: For a better result, use the prepared luminescence detection working solution immediately after mixing. The solution is stable for up to 1 hour at room temperature, but the sensitivity will decrease slightly.

3. Remove the membrane with flat tweezers from solution, blot excess liquid with an absorbent towel by gently touching the lower edge of the membrane, but do not let the membrane dry completely. Pipette the working solution to the transfer membrane, and ensure that the membrane is fully covered. Then incubate the membrane for 3-5min at room temperature . It can be done in a clear plastic wrap or plastic sheet protector.

4. Remove the membrane from the luminescent working solution, blot excess liquid with an absorbent towel. Place the membrane between two pieces of plastic wrap, then start the tablet inspection or imager test.

5. Tablet inspection: place the membrane in the clip holder with the protein side facing up. Press the tablet in the dark room for 1 minute, it shows immediate development and fixing, you may adjust the tabletting time according to the result. Alternatively, observe the result by directly pressing the tablet in sequence for 30s, 1, 3, and 5 minutes to develop and fix.

6. Imager test: place the membrane in the imager, see the instrument manual for testing.

◆ Troubleshooting

1. Solution Ⅰ is the substrate and stored in light-proof reagent bottles， Solution Ⅱ is the oxidant. First use the Solution I and then use the Solution II after changing the pipette tip.

2. Steps 1-4 (see above“ Protocol ”) can be operated under lab lighting. The sensitivity of the luminescent solution may decrease slightly with prolonged exposure to intense light, which can be avoided if it is operated in a dark room. Always wear gloves or use clean forceps while handling the membrane.

3. Do not use the rusty metallic devices such as scissors, tweezers,etc. Metal oxide particles may cause granular spots on the membrane.

4. Close the bottle cap firmly immediately after using each solution. Especially Solution II, which contains the oxidant, is easier to be reduced and loss its effectiveness.

5. Make full use of the first 30 minutes of strong fluorescence in tablet pressing. The fluorescence of this product lasts for a long time and the light signal can be stable for 1-2 hours. The fluorescence is only strong within 30 minutes after the reaction starts, and then it will gradually weaken.

6. Because of the ultra-high sensitivity of the luminescent solution, recommended primary antibody concentration is 1:1,000-1:5,000 dilution (0.2-1μg/mL), recommended secondary antibody concentration is 1:20,000-1:100,000 dilution (10-50ng/mL). Higher antibody concentration will cause high background or no band, which leads to the failure of the experiment.

7. For your health and safety, always wear a lab coat and disposable gloves. Both Solution I and Solution II are harmful to the human body, take appropriate protection during operation.

8. All products are for research use only. Not for use in diagnostic procedures.