ECL Basic / ECL Enhanced / ECL Super

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1. Product Description

This product is a chemiluminescence reagent kit based on a luminol substrate, which can be catalyzed by horseradish peroxidase (HRP) to produce light emission. The kit features an optimized substrate composition and utilizes a novel high-efficiency enhancer, resulting in a luminescence intensity 30-100 times higher than that of traditional ECL developing solutions while effectively reducing background interference. By employing a new oxidizer in place of unstable hydrogen peroxide, the kit exhibits enhanced stability and can be stored at room temperature for up to one year. When the working detection solution is catalyzed by HRP, it emits fluorescence at specific wavelengths (400-450 nm), suitable for exposure on X-ray films or direct scanning with a fluorescence CCD. It is primarily designed for applications in Western blot detection and chemiluminescent immunoassay systems.

2. Buffer Preparation

Working concentration of primary antibody: 0.2-1.0 μg/mL;
Working concentration of HRP-conjugated secondary antibody: 10-500 ng/mL (adjusted according to antibody titer);
Preparation of chemiluminescent detection working solution: Solution I : Solution II = 1 : 1; use 1-2 mL working solution per 10 cm^2 transfer membrane.

Antibody Stripping Buffers

ECL Basic:0.1 M glycine, pH adjusted to 2.7 with HCl;
ECL Enhanced: 2% SDS, 0.1 M β-mercaptoethanol, 50 mM Tris-HCl, pH 7.0;
ECL Super:6 M GuHCl, 0.2% Nonidet P-40 (NP-40), 0.1 M β-mercaptoethanol, 20 mM Tris-HCl, pH 7.5.

3. Operation Steps

After completion of the transfer according to routine procedures, perform blocking, primary antibody incubation, secondary antibody incubation, and necessary membrane washing steps. According to the size of the membrane, use 1-2 mL of working solution per 10 cm^2 of membrane. Proportionally aspirate equal volumes of Solution I and Solution II, mix well to prepare the chemiluminescence detection working solution. Use blunt forceps to remove the membrane, gently touch the lower edge of the membrane to absorbent paper to remove excess liquid. Pipette the working solution onto the transfer membrane to ensure even coverage, and incubate at room temperature for 1-2 min. This step can be performed on clean plastic wrap or in a plastic box.

3.1 X-ray Film Method

1) Use blunt forceps to pick up the transfer membrane. Gently touch the lower edge of the membrane to absorbent paper to remove excess working solution, leaving a small amount remaining. Do not let the membrane dry completely. With the protein side facing up, wrap the membrane in clean plastic wrap. Gently remove any air bubbles and secure the four corners with small pieces of transparent tape, then fix it inside an X-ray film cassette.

2) In a darkroom, place an X-ray film over the wrapped membrane, close the cassette, and expose for 30 s to 1 min. Immediately develop and fix the film. Adjust the exposure time for the next film based on the exposure intensity. If background is too high, two X-ray films can be pressed simultaneously.

3.2 Fluorescence Imaging Method

1) If using a CCD camera for imaging, the membrane can be placed in the working solution. After starting the instrument, follow the instructions to remove the transfer membrane and capture the image.
2) Adjust the instrument measurement parameters according to background levels to improve the signal-to-noise ratio.

3.3 Tube-based Chemiluminescence Method
1) The chemiluminescence detection wavelength for luminol can be set around 425 nm. Either single-point measurement or averaging of multiple measurements may be selected.
2) According to the instrument requirements, add the prepared working solution to the sample tube and measure the luminescence intensity at regular intervals. Note that the luminescence of the luminol system peaks after a certain delay (30-300 s). The measurement intervals should be kept consistent for different samples.
3) The HRP-catalyzed luminol luminescence system is also strongly influenced by reaction temperature and pH. Therefore, such factors should be considered during working reagent preparation and luminescence measurement.

4. Issues and Solutions

Problem	Cause Analysis	Recommended Solution
No bands appear on the film or the signal is weak	The efficiency of membrane transfer is low	Use pre-stained molecular weight standards to judge and improve membrane transfer efficiency
	Insufficient or mismatched antigen/antibody quantity	Increase the amount of antigen/antibody or select an appropriate antigen/antibody
	There is a problem with the X-ray film	After developing, X-ray films should be transparent. If they are completely black, it indicates that they have been fully exposed and should be discarded
	There is a problem with the developer solution	You can first expose a piece of film to verify, and if there is a problem, replace it with a new one for development
	Excessive HRP in the reaction system	Dilute HRP marker
Dirty/High background on X-ray film	The concentrations of primary antibody and secondary antibody are too high	Reduce antibody concentration and prolong blocking time
	The antibody has not been cleaned thoroughly	Increase the frequency of membrane washing
strip with empty spots	The concentrations of antigen and secondary antibody are too high	Run the diluted sample again, or alternatively, after placing the mixed chromogenic solution in an ice bath, apply it to the membrane and allow rapid color development immediately
strip irregularity	The presence of bubbles during film transfer may also be due to the transfer film not having a uniform hydration agent in the box	Try to optimize the conditions during membrane transfer

5. Precautions

1) Solution I in the kit serves as the substrate and is stored in a light-proof reagent bottle, while Solution II is the oxidizer. The typical sampling order is to take the substrate Solution I first, then change the pipette tip before taking the oxidizer Solution II.
2) This kit is relatively stable and can be stored at room temperature (25°C) for over one year. For long-term storage, it is recommended to keep it at 2–8°C.
3) When using the biotin–avidin system, avoid blocking with milk, as it may lead to high background.
4) Metal oxide particles may cause granular spots on the membrane. Avoid using scissors or tweezers with rust, and consider using flat-head plastic tweezers instead.
5) Sodium azide inhibits the catalytic activity of HRP. Try to avoid using sodium azide as a preservative in buffers.
6) Steps such as blocking, washing, and incubation are time- consuming. Note that uneven friction between the membrane and plastic surfaces may cause partial loss of bands. Incubation and blocking can be performed inside plastic wrap. Ensure that the bottom of the washing container has no obvious protrusions. To distinguish the protein- bound side of the transfer membrane, you may cut a corner as a marker.

7) Different transfer membranes have varying protein adsorption capacities. Nitrocellulose membranes are relatively soft—avoid creating folds. PVDF membranes must be uniformly hydrated with methanol before use.

8) Do not wash multiple membranes in the same container, as mutual adsorption and friction can result in strong background.

9) Avoid bubbles during transfer, blocking, and incubation.

6. Related Products

Product	Cat.No.	size
ECL Basic	BK0040-01	50 mL+50 mL
	BK0040-02	250 mL+250 mL
ECL Enhanced	BK0041-01	50 mL+50 mL
	BK0041-02	250 mL+250 mL
ECL Super	BK0042-01	50 mL+50 mL