ECL Chemiluminescent Substrate Detection Kit (Ultrasensitive)

Introduction

ECL Chemiluminescent Substrate Detection Kit (Ultrasensitive) is an enhanced chemiluminescence (ECL) substrate with ultra-high sensitivity and the most sensitive chemiluminescence substrate among our ECL luminescent solutions, achieving low femtogram levels (based on HRP concentration) through HRP. Western blot detection. Its sensitivity, luminous time, and background are noticeably excellent. It has the following characteristics:

- Sensitive: Femtogram-grade protein bands can be detected on nitrocellulose or PVDF membranes when appropriate primary and secondary antibodies are used.
- Quantification: The quantifiable range of the resulting signal spans two orders of magnitude.
- Bright signal: Exposure through film or imaging system for easy image capture.
- Long signal duration: Detectable optical signal output up to 8 hours under optimized conditions.
- Stabilized reagents: Kit components can be stable at 4°C for one year and at room temperature for 6 months.
- Affordable: Formulated to be optimized for very low concentration antibody detection.

When this product substrate is used in conjunction with optimized antibody concentrations and blocking buffers, low-abundance target proteins that cannot be detected by conventional ECL substrates can be detected.

Components and Storage

Components	100 mL	500 mL
ECL Chemiluminescent Substrate Detection Kit (Ultrasensitive)-A	50 mL	250 mL
ECL Chemiluminescent Substrate Detection Kit (Ultrasensitive)-B	50 mL	250 mL
Store the components dry at 4 °C and protect from light for 12 months.		

Experimental operations

(1) Brief operation steps

Note: Optimize the concentration of antigens and antibodies. The recommended antibody dilution must be used to guarantee a positive result. Please refer to other required materials for recommended dilution ranges.

1. Perform routine electrophoresis, membrane transfer, HRP-labeled antibody or HRP-labeled nucleic acid probe incubation and membrane washing.
2. The two substrate components were mixed in a 1:1 ratio to prepare the substrate working solution.

Note: Exposure to *sunlight or any other bright light may damage the working fluid, for best results, keep this working fluid in an amber bottle and avoid prolonged exposure to any bright light. Short-term exposure to routine laboratory lighting does not damage the working fluid.*

3. Incubate the blot membrane in substrate working solution for 5 min.
4. Aspirate excess reagent. Cover this blot film with a clean plastic film.
5. Expose the blot film on X-ray film.

(2) Detailed operation steps of western blotting

1. Remove the imprinted membrane from the protein transfer apparatus, add a suitable blocking solution and incubate for 20-60 min in a greenhouse while shaking. to block non-specific protein binding sites on the membrane. *Please note: It is important to use the antibody dilutions recommended above.*
2. Remove the membrane from the blocking solution and incubate with the primary antibody working solution in a greenhouse for 1 h while shaking; Or incubate overnight at 28 °C without shaking.
3. Add a sufficient amount of wash buffer to the membrane to ensure that the buffer completely covers the membrane. Incubate ≥ 5 min with shaking, change the wash buffer and repeat this step 4-6 times. Increasing the wash buffer volume, wash times, and wash times helps reduce the background signal.

   Note: Brief rinsing of the membrane in wash buffer before incubation will improve wash efficiency.

   Please note: It is very important to use the HRP-labeled secondary antibody dilutions recommended above.
4. Incubate the HRP-labeled secondary antibody working solution with the membrane in a greenhouse for 1 h while shaking.
5. Repeat step 3 to remove unbound HRP-labeled secondary antibodies. *Note: Membranes must be thoroughly washed after incubation with HRP-labeled secondary antibodies.*
6. Mix solution A and solution B in equal proportions to prepare a working solution. Use 0.01~0.1 mL of working solution per cm2 membrane. The working fluid can be stabilized under a greenhouse for 8 hours. *Note: Exposure* to rain *or any other bright light may damage the working fluid, for best results, keep this working fluid in an amber bottle and avoid prolonged exposure to any bright light from rain. Common lighting in laboratories does not damage the working fluid.*
7. Incubate the imprinted membrane in the working solution for 5 min.
8. Remove the imprint film from the working fluid and place it in a plastic sheet or clean plastic paper (film), suck out excess liquid with a sheet of absorbent paper and carefully press out air bubbles from between the imprint and the plastic paper.
9. Place the imprint film wrapped in plastic paper (film) in a film cassette with the protein side facing up, and turn off all lights except those suitable for film exposure, such as red safety lights.

   Note: Film must be kept dry during exposure, and for best results, take the following measures:

   ** Make sure to completely remove excess substrate from the film and plastic paper.*

   ** Use gloves throughout film processing.*

   ** Do not place the imprint film on the developed film, as the chemicals on the film will weaken the signal.*
10. Place the X-ray film on top of the film. It is recommended that the first exposure be 60 seconds. The exposure time can then be adjusted to achieve the best results. The chemiluminescence reaction is most intense during the first 5-30 min after substrate incubation. This reaction can last for several hours, but the intensity decreases over time, and if the substrate is exposed after a long period of time after incubation, the exposure time may need to be extended to obtain a strong signal. If using a phosphorescent storage imaging device or CCD camera may require a longer exposure time.

    Warning: Any movement between film and film may cause an artificially non-specific signal on the film.
11. Develop the film with a suitable developer and fuser. If the signal is too strong, shorten the exposure time or strip the imprinted film and reduce the antibody concentration to retest.

Common problems and solutions

issue	Possible causes	solution
Inverted image on film (i.e. black background, white band)	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 10-fold
There are brown or yellow bands on the membrane		
The imprint glows in the darkroom		
The signal duration is less than 8 hours		
Weak or no signal	Too much HRP in the system depletes the substrate and causes the signal to decay rapidly	Dilute the HRP-labeled secondary antibody at least 10-fold
	Insufficient amount of antigen or antibody	Increase the amount of antibodies or antigens
	Low protein transfer	Optimize transfer
	Low HRP or substrate activity	See note below*
High background	Too many HRPs in the system	Dilute the HRP-labeled secondary antibody at least 10-fold
	Inadequate closure	Optimize the closing conditions
	Closed machines are not chased	Try a different blocking reagent
	Insufficient selection of polyester	Increase wash time, frequency, or wash buffer volume
	Film overexposure	Reduce exposure time or use background eliminator
	The concentration of antigen or antibody is too high	Reduce the number of antibodies or antigens
There are spots inside the protein strip	Low protein transfer efficiency	Optimize the transfer process
	The hydration of the membrane is uneven	Hydrate the membrane moderately according to the manufacturer's recommendations
	There are air bubbles between the film and the film	Remove air bubbles before film exposure
There are spots on the background on the film	Aggregates are present in HRP labeled secondary antibodies	Use a 0.2 μm filter
Non-specific bands	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 10-fold
	Nonspecific binding of proteins due to SDS	SDS is not used during inspection

*Note: *To detect system activity, prepare 1-2 mL of substrate working solution in a clean tube in a darkroom. Turn off the lamp and add 1 μL of undiluted HRP-labeled secondary antibody working solution. The solution should immediately emit blue light, and the blue light signal fades in the following few minutes.

Note

1. For best results, all components of the system must be optimized, including sample volume, primary and secondary antibody concentrations, and types of membrane and blocking reagents. Because the substrate is extremely sensitive, it is required to use much fewer samples, primary antibodies, and secondary antibodies than most commercially available substrates, usually at least 10-20 times lower.
2. This product is used at a lower antibody concentration than the antibody required to detect using precipitated colorimetric HRP substrates. To optimize antibody concentration, perform a systematic western blot analysis.
3. No single blocking reagent is optimal for all systems, so finding the most suitable blocking buffer for each western blot detection system is essential. Blocking reagents have the potential to cross-react with antibodies, resulting in non-specific signals. Blocking buffers also affect the sensitivity of the system. When switching from one substrate to

another, signal attenuation or increased background sometimes occurs, possibly because the blocking buffer is not suitable for the new detection system.

4. When using an avidin/biotin detection system, avoid using cow's milk as a blocking reagent, because cow's milk contains unquantified endogenous biotin. This results in a high background signal.
5. Ensure the volume of wash, blocking, antibody, and substrate working solutions used to ensure that the blotting membrane is completely covered with liquid throughout the experiment and that the membrane does not dry out. Increasing the amount of blocking buffer and wash buffer can reduce nonspecific signals
6. For best results, use a shaker during the incubation step.
7. Add Tween20 (final concentration 0.05-0.1%) to blocking buffer and diluted antibody solution to reduce non-specific signaling; With high-quality products such as stain removers. It is kept in ampoules with a low content of peroxides and other impurities.
8. Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP.
9. Avoid direct contact between hands and membranes, wear gloves or use clean tweezers during the experiment.
10. All equipment must be clean and free of foreign substances. Metal instruments (such as scissors) must not have visible rust. Rust can cause speckle formation and a high background.
11. The substrate working solution is stable at room temperature for 8 hours. For best results, keep the substrate working solution in an amber flask and avoid prolonged exposure to any bright light, which will not be damaged by short exposure to routine laboratory lighting.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com