ECL Chemiluminescent Substrate Detection Kit (Enhanced)

Introduction

ECL Chemiluminescent Substrate Detection Kit (Enhanced) is used to detect antibodies directly or indirectly labeled with horseradish peroxidase HRP and its associated antigens, enabling low picy (based on HRP concentration) western blot detection. Its sensitivity, luminous time, and background are noticeably excellent.

It has the following characteristics:

（1） Easy to use: It can replace ECL luminescent substrates from other companies without special optimization of operating steps.

（2） Higher sensitivity: Detects proteins at low picogram levels.

（3） Longer signal duration: Optical signals last up to 5 hours.

（4） More imaging methods: for X-ray film, CCD or laser imagers.

Components and Storage

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

Experimental operations

1. Perform the regular SDS-PAGE, transfer, and Western Blot steps. Note labeling lgG with HRP or with primary antibody-streptavidin-biotin-HRP clipping.

   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 will not damage the working fluid.

   3) Incubate the blot membrane in ECL 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. Western Blot prepared a fresh luminous working solution at the same time as the last film wash: take equal volumes of solution A and B, respectively, and mix in a clean container. It is recommended to use the working solution immediately, but it can still be used after several hours at room temperature but with a slight reduction in sensitivity.
3. Remove the membrane with forceps and drain the rinse on the filter paper without drying the membrane completely. The membrane is completely immersed in the luminescent working solution (0.125 mL luminescent working solution/^{cm2} membrane) and in full contact with the luminescent working solution. Incubate for 3 min at room temperature, ready for immediate compression exposure. Incubation times that are too long do not increase sensitivity and sometimes result in abnormal exposure bands. The essence of the luminescence process is an enzymatic reaction, and the use of too little luminescent working fluid is not conducive to the reaction, which will also lead to uneven exposure of bands on the film and a significant reduction in sensitivity. In order to achieve the purpose of saving, the film can be cut small but do not reduce the amount of luminescent solution.
4. Pick up the membrane with tweezers and drain the luminous working fluid on the filter paper. But do not wash off the luminescent solution.
5. Open the X-ray film cassette and lay a plastic wrap with an area larger than the film on the inner surface of the cassette. Attach Western Blot film to plastic wrap, fold the plastic wrap to completely wrap Western Blot film, remove air bubbles and wrinkles, and cut off excess plastic wrap at the edges. Use filter paper to suck off excess luminous working fluid. Fix the plastic wrap covering the Western Blot film in the cassette, with the protein tape facing up.
6. X-ray film is pressed in the darkroom, and the exposure time is different for a few seconds to a few minutes. Develop and rinse.

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. Step 1~5 can be operated under fluorescent lamp; However, the sensitivity of the luminescent liquid exposed to bright light for too long may be slightly reduced, and it can be avoided by moving to a darkroom operation. Wearing gloves avoids leaving handprints on the membrane.
2. Long exposures or excess protein will deepen the background and lose the linear relationship between band strength changes. Underexposed strips are blurry.
3. The bands on the membrane glow after approximately 3 min of incubation of the luminescence working solution. Strong band luminescence is visible to the naked eye in the darkroom, and low-abundance protein band luminescence is weak or even invisible to the naked eye but can expose X-ray films. The strip luminescence time cannot be judged simply by visual observation. Fluorescence, which is invisible to the naked eye, can last for hours and sensitize X-ray film, so that weak bands can be exposed for 1-10 hours. If the bands are poor after exposure, the film can be washed with wash buffer, the secondary antibody is re-incubated, and then the ECL is re-luminesced and exposed.
4. Some plastic wraps may quench fluorescence when wrapped in blotting, so high-quality plastic wrap should be selected.

5. The position and size of bands on the film can be precisely determined using the prestained protein Marker and fluorescence-autoradiography exposure tags visible to the naked eye.
6. NaN3 can inhibit HRP activity, and the use of NaN3 should be avoided for recovery of secondary antibodies, and should not exceed 0.01% if necessary.
7. This product is for scientific use only.

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