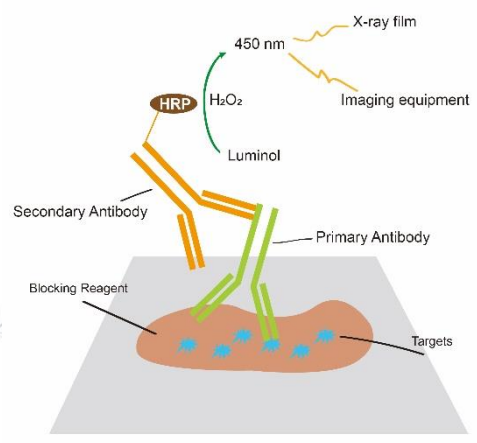
ECL Chemiluminescent Substrate Detection Kit

Introduction

The core principle of ECL reagent detection is oxidation reaction luminescence: Under alkaline conditions, lumino, the main component of the luminescent substrate, is oxidized by H_2O_2 catalyzed by horseradish peroxidase (HRP) to generate 3-aminophthalene. The excited state intermediate of the acid emits photons when it returns to the ground state. The maximum emission wavelength is 425 nm. The photon signal can be captured by X-ray film or CCD imager.

ECL Chemiluminescent Substrate Detection Kit is used to detect antibodies that directly or indirectly label horseradish peroxidase (HRP) and its associated antigens. It is often used in WB detection and chemiluminescent immunodetection systems. The principle is that the protein or nucleic acid is transferred to the blotting membrane after electrophoresis, and the primary antibody and HRP-labeled secondary antibody bind to the target protein on the membrane, or the HRP-labeled probe directly or indirectly binds to the nucleic acid on the membrane. After washing the membrane, incubate the membrane with the ECL working solution prepared by this product for several minutes at room temperature, and fix the blotting membrane with plastic wrap and fix it on the X-ray exposure cassette. Then transfer to the dark room and press the X-ray film on the film to expose for several seconds to several hours. After development and fixation, the protein or nucleic acid bands can be clearly displayed on the X-ray film. It is also possible to directly scan the blot film without X-ray film exposure.



Components and Storage

Catalog No.	Name	Size
K1129	ECL Chemiluminescent Substrate Detection Kit	100 mL (50 mL each for A and B)
Store at 2-8°C protected from light for two years.		

Protocols

1. Take X-ray film as an example:

(1) Perform conventional electrophoresis, membrane transfer, HRP-labeled antibody or HRP-labeled nucleic acid probe incubation, and membrane washing.

Note: ECL luminescent solution is the chromogenic substrate of HRP, so the final detection system must be based on HRP enzyme-labeled antibodies or nucleic acid probes.

(2) When washing the membrane for the last time, prepare a fresh luminescence working solution (100-200 μL luminescence solution/cm^2 film is recommended): Take equal volumes of A and B and mix them for later use.

Note: Different pipette tips must be used to take liquid A and liquid B; it is also recommended to use the working liquid immediately. It can still be used after a few hours at room temperature, but the sensitivity is slightly reduced.

(3) Take out the membrane with flat-tipped tweezers and place it on the filter paper to drain the dry-cleaning solution. Do not let the membrane dry completely. Use a pipette to add the working fluid to the membrane to make full contact. Incubate for 1-2 min at room temperature, ready to press the tablet immediately for exposure.

(4) Pick up the membrane with flat-tipped tweezers, and gently touch the absorbent paper with the bottom edge of the membrane to remove excess liquid on the membrane, leaving a small amount of working fluid, and do not let the membrane dry completely.

(5) Spread a piece of plastic wrap with an area larger than the film on the inner surface of the X-ray film cassette. Stick the blotting film on the plastic wrap, fold the plastic wrap to completely wrap the blotting membrane, remove bubbles and wrinkles, and cut off the excess plastic wrap at the edge. Use filter paper to absorb excess luminescent working fluid. Fix the fresh-keeping film covering the blotting membrane in the cassette with tape, with the protein band facing up.

(6) Take a piece of X-ray film in the darkroom and place it on the wrapped film, press the film, expose for 30 s-2 min, develop, fix, and rinse.

Note: The exposure time needs to be adjusted according to the exposure intensity. If the background is too high, two X-ray films can be used at the same time.

2. Other exposure methods:

If you use CCD to take pictures: first place the membrane on the platform, use white light to shoot the Marker in the imaging system, and adjust the focus, size, and exposure. Then mix A and B liquids to form a working liquid, and drop them on the membrane to ensure that every part of the membrane is covered. Choose a single exposure or multiple exposures according to your experimental method. You can choose the appropriate exposure time by yourself, or use automatic exposure. Then merge the Marker and the strip to save the image. After after the exposure，the membrane is recycled or discarded, and the platform is cleaned with ddH_2O.

Note

(1) The transfer, sealing, and incubation all need to avoid air bubbles. In addition, wearing gloves can avoid leaving fingerprints on the membrane and keep the membrane clean.

(2) Long-term exposure or excessive protein will deepen the background and make the band intensity change lose the linear relationship. Underexposure will blur the bands.

(3) Some plastic wrap may quench the fluorescence when wrapping the blotting membrane, so high-quality plastic wrap should be selected.

(4) Avoid placing multiple membranes in the same membrane washing box to wash membranes. Mutual absorption or friction may cause a deep background.

(5) Pre-stained protein Marker and fluorescence-autoradiography exposure label can be used to accurately determine the position and size of the band on the film.

(6) Use the biotin-avidin system and avoid using milk for blocking, which may cause the background to be too high.

(7) Metal oxide particles may cause granular spots on the membrane. Avoid using rusty scissors and tweezers. It is recommended to use plastic flat-tipped tweezers.

(8) Sodium azide (NaN3) can inhibit the activity of HRP. If you recover HRP-labeled probes or antibodies, you should avoid using NaN3, if necessary, do not use more than 0.01%.

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