Hypersensitive ECL Chemiluminescent Substrate

Catalog# BWR1064

Size: 100 ml (Ready to use)

Lot # Check on the product label

Introduction

This substrate is an enhanced chemiluminescent substrate with high sensitivity and unique chemiluminescent system. With low background, good stability, sensitive and enhanced signal, this reagent is good for detecting direct and indirect conjugated HRP antibodies and their related antigens, and can detect the target protein sensitively. Catalyzed by HRP, this chemiluminescent substrate can be used to expose X-ray film, and do luminometer detection directly or fluorescence CCD scan.

Kit Components

Components	Size
Hypersensitive ECL Chemiluminescent Substrate A (Reagent A)	50 ml
Hypersensitive ECL Chemiluminescent Substrate B (Reagent B)	50 ml

Storage

Store at 4℃ in dark for one year for frequent use. Or store at -20℃ for long term.

Protocol

1. Proceed the routine SDS-PAGE, transfer membrane and Western Blot steps.
2. Blot membrane: wash membrane with TBS-T twice, 10 min each. Immerse membrane into 5% defatted milk blocking solution, shake by a shaker, and incubate at room temperature for 1 hour.
3. Remove the blocking solution, incubate the membrane in properly diluted primary antibody at room temperature for 2 hours, or at 4℃ overnight on a shaker.

   Note: Dilute the primary antibody with blocking solution at the antibody manufacturer's recommended dilution.
4. Wash the membrane with 4× or 6× TBS-T for 3 times, 10 min each. Increase the volume of wash buffer, and / or the wash times can reduce the background.

   Note: Before proceeding Step 4, rinse the membrane with TBS-T simply, the wash efficiency will be increased.
5. Incubate the membrane in properly diluted HRP conjugated secondary antibody at room temperature for 2 hours on a shaker.

   Note: Dilute the secondary antibody at the antibody manufacturer's recommended dilution.
6. Repeat Step 4 to remove the non-specific conjugation of HRP conjugated secondary antibody.

Chongqing Biospes Co., Ltd Tel: +86-23-67567091 Fax: +86-23-67745923
7F, Bldg B, High-tech Venture Park, # 107 Erlang Chuangye Rd, Jiulongpo District, Chongqing, 400039, China
www.biospes.com

Note: Wash the membrane thoroughly after proceeding Step 5.

7. Prepare substrate working solution: mix Reagent A with Reagent B at ratio 1:1, i.e. Mix 1 ml of Reagent A with1 ml of Reagent B, mix thoroughly for use. The dosage of this working solution should be determined according to the membrane size, it is better to immerse the membrane completely in this working solution, i.e. Per 10 cm^2 membrane need about 1 ml of this working solution.
8. Put the membrane on a flat (protein side upwards), add the substrate working solution.
9. Incubate the membrane with substrate working solution for 1-5 min.

Note: Observe the incubation in the dark room to determine whether need to do film exposure.

10. Spread a plastic wrap or transparent glassine on the membrane, if the plastic wrap or transparent glassine is big enough, it is unnecessary to remove the excess working solution, but just need slightly press out the bubbles.
11. In the dark room, carefully put a piece of X-ray film on top of the membrane, expose 5 seconds to 1 minute, develop and fix film immediately.

Note: The exposure time can be varied according to chemiluminescence idensity to achieve optimal results. If the signal is weak, prolong the exposure time to hours.

Or use a gel visualization or a CCD camera to record chemiluminescent images of the membrane directly.

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