Hypersensitive ECL Chemiluminescent Substrate

Catalogue No.:abx090658

Hypersensitive ECL Chemiluminescent Substrate is an enhanced chemiluminescent substrate with high sensitivity and unique chemiluminescent system. With low background, good stability and enhanced signal, this reagent can be used for detecting direct and indirect conjugated HRP antibodies and their related antigens, and can detect the target protein with high sensitivity. Catalysed by HRP, this chemiluminescent substrate can be used to expose x-ray film, carry out direct luminometer detection or fluorescence CCD scan.

The 100 ml size is provided as two vials:

- Reagent A: 50 ml
- Reagent B: 50 ml

Tested Applications:	WB
Form:	Liquid
Storage:	Store in the dark at 4 °C for up to one year, or at -20 °C for long-term storage.
Note:	THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use:

Suggested Procedure

1. Carry out the SDS-PAGE, membrane transfer and Western blot steps.
2. Blot membrane: Wash the membrane twice with TBS-T. Submerge the membrane in 5% non-fat milk (blocking solution). Mix using an orbital shaker at room temperature for 1 hour.
3. Remove the blocking solution. Dilute the primary antibody with blocking solution to the appropriate dilution, then add to the membrane. Mix using an orbital shaker at room temperature for 2 hours, or overnight at 4 °C.
4. Rinse with 1X TBS-T. Wash the membrane three times with 4X or 6X TBS-T, 10 minutes per wash.
5. Dilute the secondary antibody to the appropriate dilution, then add to the membrane. Mix using an orbital shaker at room temperature for 2 hours.
6. Wash the membrane thoroughly three times with 4X or 6X TBS-T, 10 minutes per wash, to remove non-specific binding of HRP-conjugated secondary antibody.
7. Prepare the working substrate solution by mixing Reagent A with Reagent B in 1:1 ratio (e.g. add 1 ml Reagent A and 1 ml Reagent B, then mix thoroughly). Approximately 1 ml of working substrate solution is required per 10 cm^2 of membrane. The volume of working substrate solution used should be sufficiently large enough to fully cover the membrane when it is submerged.
8. Place the membrane on a flat surface (protein side facing upwards). Add working substrate solution to the membrane.
9. Allow to stand for 1-5 minutes. This could be observed in a dark room to determine whether film exposure is required.
10. Cover the membrane with plastic wrap or glass covering. Ensure that there are no bubbles present. Excess working solution may need to be removed if the covering is not sufficiently large enough.
11. In a dark room, carefully place a piece of X-ray film on top of the membrane. Expose for 5 seconds to 1 minute, then develop and fix the film immediately. The exposure time can be varied according to chemiluminescence intensity to achieve optimum results. If the signal is weak, the exposure time can be increased to a few hours. Alternatively, gel visualisation or a CCD camera can be used to record chemiluminescent images of the membrane directly.

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