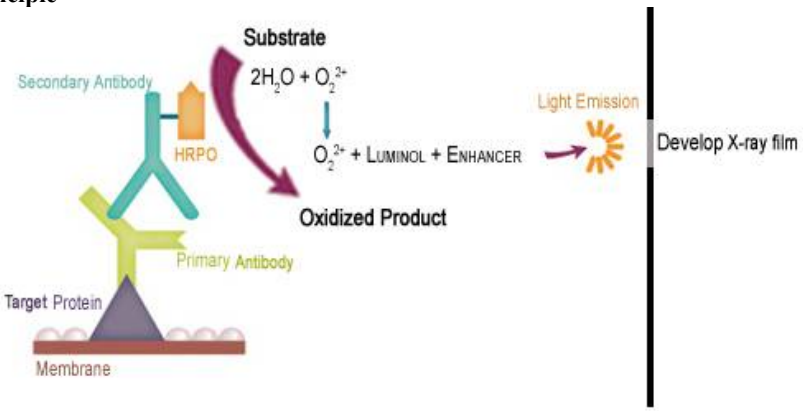
PowerOpti-ECL

Western blotting detection reagent Version 2.0

Catalog No. : LR 01-01, LR01-02

Description PowerOpti-ECL Western Blotting Detection Reagents are enhanced chemiluminescent reagents for Western blotting that uses enhanced luminol-based detection which is suitable for Western blotting assays.

Principle



Chemiluminescence is the detectable light emissions of a chemical reaction, usually using HRPO (Horse Radish Peroxidase) as the enzyme conjugated to the secondary antibody and Luminol as the substrate. Light signals can be detected by X-ray film or CCD. The Luminol is oxidized by HRPO, causing light emission from the excited state with the light emission enhanced by an enhancer for better detection by the western blotting assay.

Materials Provided

Cat No.	Solution A	Solution B
LR 01-01	100 ml	100 ml
LR 01-02	250 ml	250 ml

How to Use

1. Mix an equal volume of solution A with solution B allowing sufficient total volume to cover the membrane.
2. Drain the excess wash buffer from the washed membranes and place them, protein side up, on a suitable clean surface.
3. Incubate for one minute at room temperature.
4. Drain off excess mixed solution by holding the membrane gently with forceps and touching the edge against a tissue.
5. Place the wrapped blots, protein side up, in an X-ray film cassette.
6. Place a sheet of autoradiography film on top of the membrane. Close the cassette and expose for 15 ~ 30 seconds.
7. Remove and develop the film.

Storage Store at 2~8℃

Stability opened reagents

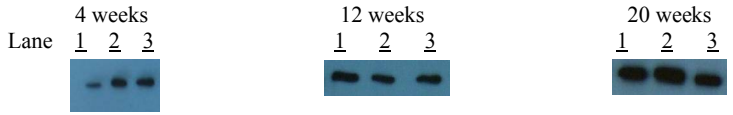
Reagent	State	Storage	Stability
Solution A	Not diluted	2~8℃	Expiry Date of kit
Solution B	Not diluted	2~8℃	Expiry Date of kit
Mixed solution	1:1 diluted	18~25℃ 2~8℃	60 minutes Use immediately

Shelf life 12 months from the manufacturing date (see printed Exp. Date on the outer package)

Remarks It is strongly recommended that the membrane incubated with PowerOpti-ECL should be exposed to X-ray film for development

Characteristics

1. Stability



(Lane 1: Company A, Lane 2 : Company B, Lane 3: PowerOpti-ECL)

After SDS-PAGE of the protein with 1 well comb and transfered to an NC membrane,

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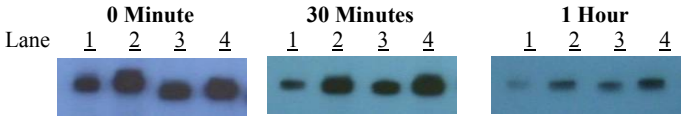
it was blotted by slicing to the same size. The stability was valid for 20 weeks storage at a temperature of 4℃

2. Signal duration



(Lane 1: Company A, Lane 2 : Company B, Lane 3: Company C, Lane 4 : PowerOpti-ECL)

After incubation of the membranes with 3 other company's western blotting detection reagents and PowerOpti-ECL, the membranes were exposed to the X-ray film at 30 minute time intervals. The results showed the light signal of the PowerOpti-ECL remained stronger when compared with the other companies reagents. The PowerOpti-ECL is Pico ~ Femto gram detectable

3. Activity duration

(Lane 1: Company A, Lane 2 : Company B, Lane 3: Company C, Lane 4 : PowerOpti-ECL)

After storing the western blotting detection reagents at 4℃, the membranes were incubated with 3 other companies western blotting detection reagents and PowerOpti-ECL. These membranes were exposed to the X-ray film at 30 minute time intervals and the result showed the light signal activity of the PowerOpti-ECL remained longer than the reagents from the other companies.

General Protocol of Western Blotting

1. Perform electrophoresis and blotting according to normal techniques. Protein should be transferred to Nucleocellulose or PVDF membrane.
2. Block non-specific binding sites by immersing the membrane in 5% non-fat dried milk, PBS or PBST for one hour at room temperature on an orbital shaker.
3. Wash the membrane for 3×10 minutes in PBST at room temperature using large volumes of washing buffer.
4. Dilute the primary antibody in PBS, PBST or 5% non-fat dried milk. Incubate the membrane in diluted primary antibody for one hour at room temperature on an orbital shaker. The dilution factor should be determined empirically for each antibody.
5. Wash the membrane for 3×10 minutes in PBST at room temperature using large volumes of washing buffer.
6. Dilute the HRP labeled secondary antibody in PBS, PBST or 5% non-fat dried milk. Incubate the membrane in diluted secondary antibody for one hour at room temperature on an orbital shaker. The dilution factor should be determined empirically for each antibody.
7. Wash the membrane for 3×10 minutes in PBST at room temperature using large volumes of washing buffer.
8. Mix an equal volume of solution A with solution B allowing sufficient total volume to cover the membrane.
9. Drain the excess wash buffer from the washed membranes and place them, protein side up, on a suitable clean surface.
10. Incubate for one minute at room temperature.
11. Drain off excess mixed solution by holding the membrane gently with forceps and touching the edge against a tissue.
12. Place the wrapped blots, protein side up, in an X-ray film cassette.
13. Place a sheet of autoradiography film on top of the membrane. Close the cassette and expose for 15 ~ 30 seconds.
14. Remove and develop the film.

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