INSTRUCTIONS

Pico Signal Western ECL Substrate

Package Contents: 2 bottles

Pico Signal Western ECL Substrate					
Catalog No.	Package	Price	Quantity/Unit	Form	Sipping and Storage Guidelines
R-ECLPF01	100 ml	73	2 bottles.	Liquid.	Shipped at 4°C. Stable for 2 years at 4 °C and 6 months at room temperature with protection from light. To avoid repeated thawing and freezing, keep the kit at 4 °C for frequent use.
R-ECLPF02	200 ml	122			
R-ECLPF03	500 ml	224			

Storage

Upon receipt, store the reagents at 4°C. When stored at room temperature, substrate components are stable for six months. Products are shipped at ambient temperature.

DESCRIPTION

The Pico Signal Western ECL Substrate is a hypersensitive ECL chemiluminescence kit produced by Genemedi for the detection of horseradish peroxidase (HRP) on immunoblots. It enables femtogram detection of antigen, which can be visualized on X-ray film or an imaging system.

PROCEDURE

1. After completing SDS-PAGE electrophoresis and protein transmembrane, remove membrane from the transfer apparatus and block nonspecific sites with Blocking Reagent for 20 - 60 minutes at room temperature (RT) with shaking. For best results, block for 1 hour at RT.

2. Remove the Blocking Reagent and add the primary antibody with appropriate dilution ratio. For best results, blot needs to be incubated with primary antibody overnight at 2-8°C.

3. Wash membranes by suspending in wash buffer and shaking for ≥ 5 minutes. Replace wash buffer at least 4-6 times. Increasing the wash buffer volume and/or the number of washes may help reduce background.

4. Incubate blot with the appropriate HRP-conjugate (secondary antibody) dilution for 1 hour at RT with shaking.

5. Repeat Step 3 to remove nonbounded HRP-conjugate.

6. Prepare working solution by mixing equal parts of Solution A and B.

Note:

The working solution can be placed at room temperature just in short time.

7. Incubate blot with working solution for about 5 minutes.

8. Remove blot from Working Solution and place it in a plastic membrane protector. (A plastic sheet protector works very well, although plastic wrap may also be used.) Use an absorbent tissue to remove excess liquid and carefully press out any bubbles from the interspace between the blot and the surface of the membrane protector.

9. Place the membrane in a film cassette with the protein side facing up. Turn off all lights except those appropriate for X-ray film exposure (e.g., a red safelight).

Note:

Film must remain dry during exposure. For optimal results, perform the following precautions:

• *Remove excess substrate from the membrane and the membrane protector.*

• *Use gloves during the entire film-handling process.*

• *Never place a blot on developed film. There may be chemicals on the film that will reduce signal.*

• *Avoid contacting the membrane, especially on the side with the protein bands.*

10. Carefully place film on top of the membrane. A recommended first exposure time is 60 seconds; then the exposure time can be optimized to achieve optimal results.

Note:

If the signal is too intense, reduce the exposure and/or development incubation times or optimize the system by decreasing the antigen and/or antibody concentrations.

On an optimized blot, light emission continues for 8 hours after substrate incubation and may decrease with time. Longer exposure time may be necessary as the blot ages.

CAUTIONS

1. Switch tips between substrate A and B, cross contamination between two substrates will accelerate their loss of efficacy.

2. To avoid reduction of substrate B (contain oxidant), please screw the caps every time after usage.

3. Exposure to the sun or any other intense light may destroy the substrate. For best results, please keep the substrate working solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not destroy the working solution.

4. Since no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for relevant Western blot system. Identifying the proper blocking buffer can help increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent.

Contact Information

Genemedi Biotech. Inc.

For more information about reagents, please visit: https://www.genemedi.net/i/reagent
For more information about Genemedi products and to download manuals in PDF format, please visit our web site: www.genemedi.net
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