PRODUCT DATA SHEET

Biodlight™ Western Chemiluminescent HRP Substrate

Background:

Chemiluminescent detection systems have emerged as the best all-around method for detection of Western blots. They eliminate the hazards associated with radioactive materials and toxic chromogenic substrates.The speed and sensitivity of these methods are unequalled by traditional alternatives. Horseradish peroxidase (HRP) conjugated secondary antibodies are utilized in conjunction with specific chemiluminescent substrates to generate the light signal. Horseradish peroxidase-antibody conjugates have a very high turnover rate, giving good sensitivity with short reaction times.

Introduction:

Biodlight Western HRP Substrate provides high sensitivity in western or dot/slot/spot blotting applications on both PVDF and nitrocellulose transfer membranes,and is compatible with all commonly used buffers and blocking reagents.The HRP substrate consists of Luminol Reagent and Perixide Solution.Working HRP substrate is prepared by combining equal volumes of Luminol Reagent and Perixide Solution.The HRP substrate produces a high intensity signal with low background for detection of both high and low abundance.

Contents:

Cat No.: BLH01S020	Cat No.: BLH01S050
Luminol Reagent,10ml	Luminol Reagent,25ml
Peroxide Solution, 10ml	Peroxide Solution, 25ml

Detailed Western Blotting Procedure:

1. Remove blot 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 appropriate primary antibody dilution. Incubate blot for 1 hour with shaking.If desired, blots may be incubated with primary antibody overnight at 2-8 ℃.

3. Wash membrane by suspending it in Wash Buffer and agitating 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.Briefly rinsing membrane in wash buffer before incubation will increase wash efficiency.

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

5.Repeat Step 3 to remove non-bound HRP-conjugate.Membrane must be thoroughly washed after incubation with the HRP-conjugate.

6.Prepare Working Solution by mixing equal parts of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Use 0.1 ml Working Solution per cm2 of membrane. The Working Solution is stable for 8 hours at room temperature.Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working

Solution in an amber bottle and avoid prolonged exposure to any intense light. Typical laboratory lighting will not harm the Working Solution.

7.Incubate blot with Working Solution for 5 minutes.

8.Remove blot from Working Solution and place it in a plastic membrane protector; a plastic sheet

protector or plastic wrap may be used. Use an absorbent tissue to remove excess liquid and to carefully press out any bubbles from between the blot and surface of the membrane protector.

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

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

Make sure excess substrate is removed from the membrane and the membrane protector.

Use gloves during the entire film-handling process.

Never place a blot on developed film, as there may be chemicals on the film that will reduce signal.

10.Carefully place a piece of film on top of the membrane. A recommended first exposure time is 60 seconds. Exposure time may be varied to achieve optimal results. Enhanced or pre-flashed film is not necessary.Light emission is intense and any movement between the film and membrane can cause artifacts on the film.The exposure time may be varied to achieve optimal results. If the signal is too intense, reduce exposure time or optimize the system by decreasing the antigen and/or antibody concentrations.Light emission is most intense during the first 5-30 minutes after substrate incubation. Light emission will continue for several hours, but will decrease with time. Longer exposure times may be necessary as the blot ages.

11.Develop film using appropriate developing solution and fixative. Blot may be stripped and reprobed if necessary.

Troubleshooting:

Problem	Possible Cause	Solution
High background	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
	Inadequate blocking	Optimize blocking conditions
	Inappropriate blocking reagent	Try a different blocking reagent
	Inadequate washing	Increase length, number or volume of washes
	Concentration of antigen or antibody is too high	Decrease amount of antigen or antibody
	Protein-protein interactions	Use Tween-20(0.05-0.1%) surfactant in the wash and detection solutions to minimize protein-protein interactions and increase the signal-to-npise ratio
	Film has been overexposed	Shorten exposure time.Initial exposure of 30 seconds is recommended.
Weak or no signal	Too much HRP in the system depleted the substrate and caused the signal to fade quickly	Dilute HRP-conjugate at least 10-fold
	Insufficient quantities of antigen or antibody	Increase amount of antibody or antigen
	Inefficient protein transfer	Optimize transfer
	Azide inhibits HRP	Do not use azide in the blotting solutions.
Speckled background	Aggregate formation in the HRP- conjugate	Filter conjugate through a 0.2 m filter
Non-specific bands	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
	SDS caused nonspecific binding to protein bands	Do not use SDS during immunoassay procedure
Reverse images on film	Too much HRP-conjugated antibody	Reduce concentration of secondary, HRP-conjugated antibody.

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