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Product Manual

SuperBright MaxiSignal ECL Substrate

61088 61089

Number	Description
61088	**SuperBright MaxiSignal ECL Substrate,** sufficient for 200cm^2 of membrane **Kit Contents:** **SuperBright MaxiSignal Solution A,** 10mL **SuperBright MaxiSignal Solution B,** 10mL
61089	**SuperBright MaxiSignal ECL Substrate,** sufficient for 1000cm^2 of membrane **Kit Contents:** **SuperBright MaxiSignal Solution A,** 50mL **SuperBright MaxiSignal Solution B,** 50mL

Storage: Upon receipt store solutions at 4°C. When stored at room temperature, substrate components are stable for six months. These products are shipped at ambient temperature.

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Introduction

The SuperBright MaxiSignal ECL Substrate is an extremely sensitive enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) on immunoblots. This substrate's extremely intense signal output enables detection of low femtogram amounts of antigen. Furthermore, this substrate requires substantial dilutions of the primary and secondary antibodies. The sensitivity, intensity and duration of the signal allow for easy detection of HRP using photographic or other imaging methods. Blots also can be repeatedly exposed to film to obtain optimal results or stripped of the immunodetection reagents and reprobed.

Important Product Information

- For best results, it is ESSENTIAL to optimize all components of the system including sample amount, primary and secondary antibody concentration, and the choice of membrane and blocking reagents. Because the substrate is extremely sensitive, SuperBright MaxiSignal ECL Substrate requires using much less sample and primary and secondary antibodies than all other commercially available substrates.
- The antibody concentrations required are much more dilute than those used with precipitating colorimetric HRP systems and our competitors' similar products. To optimize the appropriate concentrations, perform a systematic dot blot analysis.
- Because no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for each Western blot system. Determining the proper blocking buffer can help increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent. Furthermore, when switching from one substrate to another, a diminished signal or increased background sometimes results because the blocking buffer was not optimal for the new system.
- Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenous biotin, which will result in high background.
- Use a sufficient volume of wash buffer, blocking buffer, antibody solution and Substrate Working Solution to cover blot and ensure that it never becomes dry. Using large blocking and wash buffer volumes may reduce nonspecific signal.
- For optimal results, use a shaking platform during incubation steps.
- Add Tween®-20 (final concentration of 0.05%) to the blocking buffer and when preparing all antibody dilutions to reduce nonspecific signal.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
- Do not handle membrane with bare hands. Always wear gloves or use clean forceps.
- All equipment must be clean and free of foreign material. Metallic devices (e.g., scissors) must have no visible signs of rust. Rust may cause speckling and/or high background.
- The Substrate 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. Short-term exposure to laboratory lighting will not harm the Working Solution.

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Procedure Summary

Note: The recommended range of antibody dilutions must be used to obtain positive results. The optimal antigen and antibody amounts to use may require experimentation.

1. Dilute primary antibody to 5-100ng/mL.
2. Dilute secondary antibody to 2-10ng/mL.
3. Mix the two substrate components at a 1:1 ratio to prepare the substrate Working Solution.

   Note: 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. Short-term exposure to laboratory lighting will not harm the Working Solution.
4. Incubate blot 5 minutes in SuperBright MaxiSignal ECL Substrate Working Solution.
5. Drain excess reagent. Cover blot with clear plastic wrap.
6. Expose blot to X-ray film or Use imaging devices.

Additional Materials Required

- **Completed Western blot membrane**: Use any suitable protocol to separate proteins by electrophoresis and transfer them to a membrane.
- **Dilution Buffer**: Use either Tris-buffered Saline (TBS) or Phosphate-buffered Saline (PBS).
- **Wash Buffer**: Add 5mL of 10% Tween-20 to 1000mL Dilution Buffer. (The final concentration of Tween-20 will be 0.05%.)
- **Blocking Reagent**: Add 0.5 ml of 10% Tween-20 to 100mL of a blocking buffer. Choose a blocking buffer with the same base component as the Dilution Buffer.
- **Primary Antibody**: Choose an antibody that is specific to the target protein(s). Use the Blocking Reagent to prepare a primary antibody working dilution ranging from **5ng/mL** to **100ng/mL**. For example, if the primary antibody is supplied at 1mg/mL, dilute it in the range from **1:10,000** to **1:200,000**. The necessary dilution to use depends on the specific primary antibody and the amount of antigen on the membrane and will require optimization for each experimental system.
- **Secondary Antibody:** Use the Blocking Reagent to prepare a HRP-conjugate working dilution ranging from **2ng/mL** to **10ng/mL**. For example, if the antibody is supplied at 1mg/mL, dilute it in the range from **1:100,000** to **1:500,000**. The necessary dilution varies depending on the primary antibody, HRP-conjugate and amount of antigen on the membrane and will require optimization for each experimental system.
- **Film cassette, developing and fixing reagents**: For processing autoradiographic film.

  or
- **Imaging devices:** e.g., Bio-Rad's Molecular Imager System or a CCD Camera.
- **Rotary platform shaker**: For agitation of membrane during incubations.

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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.

*******Please Note: It is critical to use the recommended antibody dilution indicated in the Additional Materials Required section.**

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°C.
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.

   Note: Briefly rinsing the membrane in wash buffer before incubation increases wash efficiency.

*******Please Note: It is critical to use the recommended HRP-conjugate dilution indicated in the Additional Materials Required section.**

4. Incubate blot with the appropriate HRP-conjugate (secondary antibody) dilution for 1 hour at RT with shaking.
5. Repeat Step 3 to remove nonbound HRP-conjugate.

   Note: Membrane MUST be thoroughly washed after incubation with the HRP-conjugate.
6. Prepare Working Solution by mixing equal parts of the MaxiSignal Solution A and the MaxiSignal solution B. Use 0.1mL Working Solution per cm^2 of membrane. The Working Solution is stable for 8 hours at room temperature.

   Note: 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 works very well, although plastic wrap may also be used.) Use an absorbent tissue to remove excess liquid and to carefully press out any bubbles from between the blot and the 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).

   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.
10. Carefully place film on top of the membrane. A recommended first exposure time is 60 seconds; however, exposure time can be varied to achieve optimal results. Enhanced or pre-flashed autoradiographic film is not necessary.

    Caution: Light emission is intense and any movement of the film or membrane can cause artifacts on the film.

    Note: After the film has been developed, the exposure time may be varied to achieve optimal results. If the signal is too intense, reduce the exposure and 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 will decrease with time. Longer exposure times may be necessary as the blot ages.

    If using a storage phosphor imaging device (e.g., Bio-Rad's Molecular Imager System) or a CCD Camera (e.g., Cell Bioscience's ChemiImager System), longer exposure times may be necessary.
11. Develop film using appropriate developing solution and fixative. Blot may be stripped and reprobed if necessary.

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Troubleshooting

Problem	Possible Cause	Solution
Reverse image on film (i.e., white bands with a black background)	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
Membrane has brown or yellow bands		
Blot glows in the darkroom		
Signal duration is less than 8 hours		
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
	Reduction of HRP or substrate activity	**See note below
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
	Film has been overexposed	Decrease exposure time
	Concentration of antigen or antibody is too high	Decrease amount of antigen or antibody
	Poor antibody specificity	Try different antibodies.
Spots within the protein bands	Inefficient protein transfer	Optimize transfer procedure
	Unevenly hydrated membrane	Perform manufacturer's recommendations for hydrating membrane properly
	Bubble between the film and the membrane	Remove all bubbles before exposing blot to film
Speckled background on film	Aggregate formation in the HRP-conjugate	Filter conjugate through a 0.2µm filter
Nonspecific 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
	Poor antibody specificity	Try different antibodies.

**To test the activity of the system in the darkroom, prepare 1-2mL of the SuperBright MaxiSignal Substrate Working Solution in a clear test tube. With the lights turned off, add 1µL undiluted HRP-conjugate to the Working Solution. The solution should immediately emit a blue light that will fade over the next several minutes.

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