ECL Plus Western Blotting Substrate

Catalog number: AR1196-200

Boster's ECL Plus Western Blotting Substrate is an ultra-sensitive, luminol-based enhanced chemiluminescent (ECL) substrate for the detection of horseradish peroxidase (HRP) at high sensitivity levels (low picogram to mid-femtogram).

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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ECL Plus Western Blotting Substrate

Catalog Number: AR1196-200

List of Components

Description	Quantity	Volume	Catalog Number
Reagent A: Luminol and luminous enhancer	1	100mL	AR1196-200-A
Reagent B: Peroxide and stabilizer	1	100mL	AR1196-200-B

Overview

Product Name	ECL Plus Western Blotting Substrate
SKU/Catalog Number	AR1196-200
Form Supplied	Reagent A and B: ready-to-use 1X solutions
Pack Size	200mL(sufficient reagents for 2000 cm^2 of membrane)
Storage	Upon receipt store ECL Plus Western Blotting Substrate at 4°C. Protect from light. It is stable at 4°C for one year.
Detection Method	Chemiluminescent
Substrate Type	HRP (Horseradish Peroxidase) Substrate
Description	Boster's ECL Plus Western Blotting Substrate is an ultra-sensitive, luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) at high sensitivity levels (low picogram to mid-femtogram).
Equivalent	Thermofisher Pierce™ ECL Plus Western Blotting Substrate (Product No. 332132, 32124), SuperSignal™ West Pico PLUS Chemiluminescent Substrate (34577, 34578, 34579, 34580); Bio-Rad Immu-Star™ WesternC™ Chemiluminescent Kit (Product No. 170-5070)
Cite This Product	ECL Plus Western Blotting Substrate (Boster Biological Technology, Pleasanton CA, USA, Catalog # AR1196-200)
Application	To detect antigen at low-picogram to mid-femtogram level by reacting with horseradish peroxidase *Our Boster Guarantee covers the use of this product in the above tested applications.

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Notes:

Type of ECL Western Blotting Substrate	Sensitivity Level	Catalog Number
Hypersensitive ECL Chemiluminescent Substrate	low picogram	AR1170
ECL Plus Western Blotting Substrate	low-picogram to mid-femtogram level	AR1196-200

Assay Principle

Boster's ECL Plus Western Blotting Substrate is an ultra-sensitive, luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) at high sensitivity levels (low picogram to mid-femtogram). Boster Western Blotting Substrate may be used for immunoblots, western blots, dot blots and any blotting application utilizing horseradish peroxidase (HRP)-conjugates. The substrate can be used with various blocking buffers and on nitrocellulose or PVDF membranes. Such blots will exhibit low backgrounds. Produced chemiluminescence can be visualized on CCD imaging systems or x-ray film.

Additional Materials Required

1. Nitrocellulose or PVDF membranes.
2. X-ray film or an imaging system.
3. Rotary platform shaker for agitation of membrane during incubations.
4. Wash Buffer: PBS or TBS containing 0.05-0.1% Tween-20 (PBS: 10mM Na_3PO_4, 150mM NaCl, pH7.2; TBS: 10mM Tris, 150mM NaCl, pH7.4).
5. Blocking Buffer: Add some blocking medium (e.g. casein, BSA or non-fat milk) into the Wash Buffer for a final blocking medium concentration of 1-5% (w/v).
6. Primary Antibody: Choose an antibody specific to the target proteins. Prepare the antibody stock solution in Wash Buffer or Blocking Buffer.
7. HRP-conjugated Secondary Antibody: Choose a HRP-conjugated Secondary Antibody that specifically binds to the primary antibody.

Important Product Information

- For optimal results, use a shaking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP.
- Do not handle the membrane with bare hands. Always wear gloves or use clean forceps.
- All equipment must be clean and free of foreign material. Metallic devices must have no visible signs of rust. Rust may cause speckling and high background.
- Exposure to the sun or any other intense light can harm the substrate. For best results 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 harm the working solution.

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- Empirical testing is essential to determine the appropriate blocking reagent for each Western blot system, as crossreactivity of the blocking reagent with antibodies can cause nonspecific signal and varying system sensitivity.
- When using avidin/biotin systems, avoid using milk as a blocking reagent as milk contains variable amounts of endogenous biotin, which causes high background signal.
- Use sufficient volumes of wash buffer, blocking buffer, antibody solution and substrate working solution to cover the blot and ensure that it never becomes dry. Using large blocking and wash buffer volumes minimizes nonspecific signal.
- Add Tween™ 20 Detergent (final concentration of 0.05-0.1%) to the blocking buffer and all diluted antibody solutions to minimize nonspecific signal.
- Do not use polystyrene vessels to mix and prepare the substrate working solution; this type of plastic causes the solution to become cloudy and produce a precipitate.
- Use new pipette tips separately while pipetting reagent A and B to avoid cross contamination.

Assay Protocol

1. Remove blot from the transfer apparatus and wash membrane with Wash Buffer 3 times for 5 minutes each.
2. Block nonspecific sites with Blocking Buffer for 60 minutes at room temperature with shaking.
3. Remove the blocking buffer and add the primary antibody working solution. Incubate blot for 1 hour at room temperature with shaking or overnight at 2-8°C with shaking.
4. Suspend membrane in Wash buffer and agitate for more than 5 minutes. Replace Wash Buffer at least 4-6 times. Increasing the volume of Wash Buffer, the numbers of washes and wash duration may help minimize background signal.
5. Incubate blot with the HRP conjugated secondary antibody working solution for 1 hour at room temperature with shaking.
6. Repeat Step 3 to remove nonbound HRP conjugate.
   Note: The membrane must be thoroughly washed after incubation with the HRP conjugate.
7. Prepare the substrate working solution by mixing Reagent A and Reagent B at 1:1. Use 0.1mL working solution per cm^2 of membrane.
8. Incubate blot with working solution for 1-5 minutes at room temperature.
9. Place blot in a clear plastic wrap or transparent plastic sheet protector. Use an absorbent tissue to remove excess liquid and carefully press out any bubbles from between the blot and the membrane protector.
10. Place the protected membrane in a film cassette with the protein side facing up.
11. Place X-ray film on top of the blot membrane. Perform a exposure of 1 minute, Vary the exposure time to achieve optimal results. Light emission is most intense during the first 5-30 minutes after substrate incubation.
    Note: CCD detection: Put the blot membrane in the CCD and detect the chemiluminescence image according to the manufacturers' instructions.
12. Develop and fix the film.

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Troubleshooting

Problem	Possible Cause	Solution
Blot glows in the dark room	Too much HRP in the sysem	Dilute HRP conjugate further
Membrane has brown or yellow bands		
Signal fades quickly		
Weak or no signal	Used insufficient quantities of antigen or antibodies	Increase the amount of antigen or antibodies
	Insufficient protein transfer	Optimize transfer condition
	Low HRP or substrate activity	Replace the secondary antibody or substrate
High Background	Too much HRP in the system	Dilute HRP-conjugate further
	Inadequate blocking or used inappropriate blocking buffer	Optimize blocking conditions
	Insufficient washing	Increase duration, number and volume of washes
	Use too much antigen or antibodies	Decrease the amount of antigen or antibodies
	Use non-specific primary antibody	Replace the antibody
	Overexposed film	Decrease exposure time
Spots within the protein bands	Ineffective protein transfer	Optimize transfer procedure
	Unevenly hydrated membrane	Replace the membrane
	Bubble between X-ray film and membrane	Remove all bubbles before exposing blot to film
Speckled background	Inadequate blocking	Optimize blocking conditions
Nonspecific bands	Too much HRP in the system	Dilute HRP conjugate further
	SDS caused nonspecific binding to protein	Do not use SDS
	Use non-specific primary antibody	Replace the antibody
	Inadequate blocking	Optimize blocking conditions