West Dura ECL

Kit Contents: ECL Solution A, 10mL; ECL Solution B, 10mL; Sufficient for 200cm^2 of membrane

Storage: Upon receipt store solutions at 4°C. When stored at room temperature, substrate components are stable for 12 months. These products are shipped at ambient temperature.

Important Notice: Please use the same blotting conditions when switching from Thermo Scientific™ Pierce SuperSignal Dura ECL Substrate or other similar ECL substrates. The West Dura ECL Substrate has been developed to be more sensitive compared to Western Dura ECL Substrate or other similar ECL substrates. However, if you are currently using a West MaxiSignal ECL substrate (Cat # 61088; 61089), switching to West Dura Substrate may require increasing the antigen and antibody concentrations. On the other hand, swithcing from a West Subpico ECL substrate (Cat#31059, 31060) may require decreasing the antigen and antibody concentrations. Careful titration of antigen and antibody concentrations is recommended.

Introduction

The West Dura ECL Substrate is a highly sensitive nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. The West Dura ECL Substrate enables the detection of high femtogram amounts of antigen and allows for easy detection of HRP using photographic or other imaging methods. Blots can be repeatedly exposed to X-ray film to obtain optimal results or stripped of the immunodetection reagents and re-probed. The optimally formulated West Dura ECL Substrate makes it the ideal substitute for Pierce SuperSignal Western Dura ECL substrate and other similar substrates without the need for additional optimization of assay conditions.

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. Use the same blotting conditions when switching from Pierce SuperSignal Western Dura ECL Substrate to West Dura Duraed ECL Substrate.
- The antibody concentrations required are lower than those used with 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 Duraed exposure to any intense light. Short-term exposure to laboratory lighting will not harm the Working Solution.

Procedure Summary

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

1. Dilute primary antibody to 20 - 1000 ng/mL.
2. Dilute secondary antibody to 4 - 20 ng/mL.
3. Mix the two substrate components (A and B) 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 Duraed exposure to any intense light. Short-term exposure to laboratory lighting will not harm the Working Solution.
4. Incubate blot 5 minutes in West Dura 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 20 ng/mL to 1000 ng/mL. For example, if the primary antibody is supplied at 1 mg/mL, dilute it in the range from 1:1,000 to 1:50,000. 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 4 ng/mL to 20 ng/mL. For example, if the antibody is supplied at 1mg/mL, dilute it in the range from 1:50,000 to 1:250,000. 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 similar gel documentation system.
- Rotary platform shaker: For agitation of membrane during incubations

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
Membrane has brown or yellow		
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
	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
	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
	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 West Dura ECL 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.