IMMUNOLOGICAL SCIENCES

ECL-2003 ECL West Dura Plus Substrate

CAT.#	Description	Sizes
ECL-2003	ECL West Dura Plus Substrate	2x 50 ml (1000 cm^2)

Kit Contents:
(ECL Solution A) + (ECL Solution B)
Storage: Upon receipt store reagents at room temperature. Products are shipped at ambient temperature.

Introduction

The ECL West Dura Plus is a sensitive, luminol-based enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) on immunoblots.
ECL West Dura Substrate enables detection of ***mid femtogram*** amount of antigen by oxidizing luminol in the presence of HRP and peroxide. This reaction produces a prolonged chemiluminescence which can be visualized on X-ray film or an imaging system.

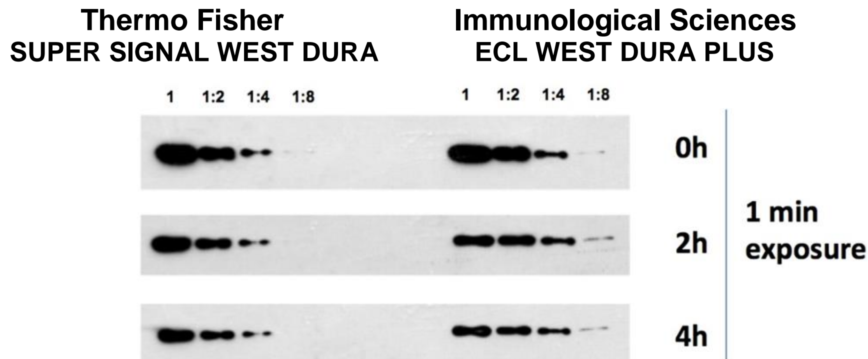
Duration of the Signal: **12～20h** if the conc. of the secondary antibody is 2–20 ng/mL

Stability of the Working solution: 3 days: (without the HRP Secondary antibody)

- Optimal signal intensity and duration can be attained with appropriate primary and secondary antibody dilutions

Antibody dilution ranges to use with ECL West Dura Plus Substrate.

Primary Antibody Dilution Range from a 1mg/mL stock	Secondary Antibody Dilution Range from a 1mg/mL stock
1:1,000-1:50,000 or 0.02-1.0μg/mL	1:50,000-1:250,000 or 4-20ng/mL



Important notes

Western blot results require optimizing the process components and steps, including sample amount, primary antibody concentration, secondary antibody concentration and incubation times, gel type, transfer method, membrane type, blocking reagent, wash buffer.

- Use a sufficient volume of all solutions to ensure membrane never becomes dry.
- For optimal results, use a shaking or rocking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers, as it inhibits HRP.
- Always wear gloves or use clean, plastic forceps. Metallic devices (e.g., scissors) must have no visible signs of rust, which may cause speckling and/or high background.
- The substrate Working Solution is stable for **8 hours** at room temperature. It can be stable **3 days** without HRP Secondary Antibody . Exposure to the sun or any other intense light can harm the Working Solution.
  Short-term exposure to laboratory lighting will not harm the Working Solution.

Additional Materials Required (not provided)

- **Western blot membrane**: Use any suitable protocol to separate proteins by electrophoresis and transfer them to a membrane.
- **X-ray film or imaging system**
- **Rotary or rocking platform shaker**: For agitation of membrane during incubations.

Procedure

Note: Western blot results require optimizing the process components and steps. See Important Product Information.

1. Primary Antibody (1mg/ml) suggested dilution: **1:1,000-1:50,000,** incubate antibody for one hour to overnight.
2. Sufficiently wash the blot with appropriate buffer.
3. Secondary Antibody (1mg/ml) suggested dilution: **1:50,000-1:250,000,** incubate for approximately 30-60 minutes.
4. Prepare Working Solution by mixing equal parts of ECL Solution A and the ECL Solution B.
   Use 0.1mL Working Solution per 1 cm^2 of membrane. The Working Solution is stable for 8 hours at room temperature.
5. Incubate the blot in Working Solution for 5 minutes.
6. Remove the blot from Working Solution and drain excess reagent.
7. Place the blot in clear plastic wrap or sheet protector and remove bubbles.
8. Expose the blot to X-ray film or imaging system.

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.
Imaging devices: Use a gel documentation system.
Rotary platform shaker: For agitation of membrane during incubations

Additional notes

➢ 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 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 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.

➢ 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.

Troubleshooting

Problem	Possible Cause	Solution
Reverse image on film (i.e., white bands with a black background)	Too much HRP in the system	Further dilute the HRP-conjugate (see guidelines in Table 1)
Membrane has brown or yellow bands		
Blot glows in the darkroom		
Signal duration is less than 24 hours		
Weak or no signal	Too much HRP in the system depleted the substrate and caused the signal to fade quickly	Further dilute the HRP-conjugate (see guidelines in Table 1)
	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	Further dilute the HRP-conjugate (see guidelines in Table 1)
	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 antibody
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 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	Further dilute the HRP-conjugate (see guidelines in Table 1)
	SDS caused nonspecific binding to protein bands	Do not use SDS during the Western blot procedure
	Poor antibody specificity	Try different antibody
	Insufficient blocking	Increase blocking time or use different blocking reagent

**To test the activity of the system in the darkroom, prepare 1-2mL of the ECL West Dura Plus 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. If no light emission is evident, test another source of HRP to determine the root cause.