ECL-2002 ECL West Femto Plus Substrate

CAT.#	Description	Sizes
ECL-2002	ECL West Femto Plus Substrate	2x50 ml (1000 cm2)

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 Femto Plus is a sensitive, luminol-based enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) on immunoblots.
ECL West Femto Substrate enables detection of ***low 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.

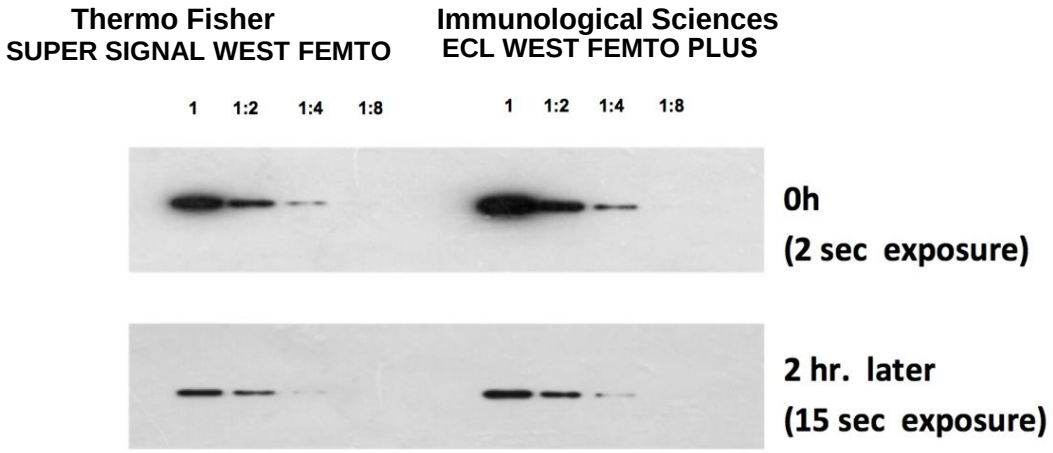
Duration of the Signal: **6～12h** if the conc. of the secondary antibody is 2–20 ng/mL

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 Femto Plus Substrate.

Primary Antibody Dilution Range from a 1mg/mL stock	Secondary Antibody Dilution Range from a 1mg/mL stock
1:10,000-1:200,000 or 5 -100ng/mL	1:100,000-1:500,000 or 2-10ng/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: **1:10,000-1:200,000,** 5 -100ng/ml, incubate antibody for one hour to overnight.
2. Sufficiently wash the blot with appropriate buffer.
3. Secondary Antibody (1mg/ml) suggested dilution: **1:100,000-1:500,000,** 2-10ng/mL ,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.

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-conj. 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 non bound HRP-conjugate.Note: Membrane MUST be thoroughly washed after incubation with the HRP-conjugate.
6. Prepare Working Solution by mixing equal parts of the ECL Solution A and the ECL solution B. Use 0.1mL Working Solution per cm2 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.

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 Femto 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.