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Enhancer Chemiluminescent (ECL) Substrate

Catalog Numbers TA0050,TA0100、TA0260

Product description

Enhancer Chemiluminescent (ECL)Substrate is a sensitive, luminol-based enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) on immunoblots. Enhancer Chemiluminescent (ECL) Substrate enables low femtogram detection of antigen by oxidizing luminol in the presence of HRP and peroxide. This reaction produces a prolonged chemiluminescence that can be visualized on X-ray film or an imaging system. Optimal signal intensity and duration can be attained with appropriate primary and secondary antibody dilutions (see Below Table).

Table Antibody dilution ranges to use with Enhancer Chemiluminescent (ECL)Substrate.

Primary antibody dilution range from a 1 mg/mL stock	Secondary antibody dilution range from a 1 mg/mL stock
1:1,000-1:1,000 or 0.1-1.0 μg/mL	1:5,000-1:20,000 or 50-200 ng/mL

Guidelines

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

Contents

Contents	Cat. No. TA0050 (for 2,000cm^2)	Cat. No. TA0100 (for 5,000cm^2)	Cat. No. TA0260 (for 10,000cm^2)	Storage
Enhancer Luminol/Enhancer Solution	50 mL	100 mL	260 mL	Room temperature
Enhancer Stable Peroxide Solution	50 mL	100 mL	260 mL	Room temperature

Materials and equipment required but not included

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

Perform Western blot

Note: Western blot results require optimizing the process components and steps. See Guidelines.

1. Incubate the blot with 0.1-1.0 μg/mL primary antibody for one hour to overnight.
2. Sufficiently wash the blot with appropriate buffer.
3. Incubate the blot with 50-200 ng/mL secondary antibody for approximately 30-60 minutes.
4. Prepare Working Solution by mixing equal parts of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Use 0.1 mL Working Solution per 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.

For Research Use Only. Not for use in diagnostic procedures.

Troubleshooting

Observation	Possible cause	Recommended action
Reverse image on film (i.e., white bands on black background).	Excess HRP in the system.	Further dilute the HRP-conjugate. Excess HRP in the
Membrane has brown or yellow bands.	system.	Further dilute the HRP-conjugate.
Blot glows in the darkroom.	Excess HRP in the system.	Further dilute the HRP-conjugate. Signal rapidly dies.
	Excess HRP in the system.	Further dilute the HRP-conjugate.
		Load less sample.
Weak or no signal.	Excess HRP in the system depleted the substrate and caused the signal to quickly fade.	Further dilute the HRP-conjugate.
	Insufficient quantities of antigen or antibody.	Increase amount of antibody or antigen.
		Use Enhancer Chemiluminescent Substrate.
	Inefficient protein transfer.	Optimize transfer.
	Reduction of HRP or substrate activity.	Test system activity in the darkroom by preparing 1-2 mL of the Substrate Working Solution in a clear test tube. With the lights off, add 1 µL 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.
High background.	Excess HRP in the system.	Further dilute the HRP-conjugate.
	Inadequate blocking.	Optimize blocking conditions.
	Inappropriate blocking agent.	Try a different blocking agent
	Inadequate washing.	Increase length, number, or volume of washes.
	Overexposed film.	Decrease exposure time
	Antigen or antibody concentration too high.	Decrease amount of antigen or antibody.
	Poor antibody specificity.	Use Enhancer Chemiluminescent Substrate
Spots within protein bands.	Inefficient protein transfer.	Optimize transfer procedure.
	Unevenly hydrated membrane.	Perform manufacturer's recommendations for properly hydrating membrane.
	Bubble between the film and membrane.	Remove bubbles before exposing blot to film.
Speckled background on film.	Aggregate formation in the HRP-conjugate.	Filter conjugate through a 0.2 µm filter.
Nonspecific bands.	Excess HRP in the system.	Further dilute the HRP-conjugate.
	SDS caused nonspecific binding to protein bands.	Do not use SDS during the Western blot procedure.
	Poor antibody specificity.	Use Enhancer Chemiluminescent Substrate Insufficient
	blocking.	Increase blocking time or use different blocking reagent.