eECL Western Blot Kit

E665966

Store at 2-8℃ protect from light.

Introduction:

eECL Western Blot Kit is an enhanced chemiluminescent (ECL) horseradish peroxidase (HRP) substrate that enables picogram to high femtogram-level protein detection by western blot analysis. eECL Western Blot Kit is designed to provide excellent signal intensity and pictogram to femtogram sensitivity for western blotting with HRP conjugates. The intensity of the light emission combined with the exceptional duration allows for the acquisition of multiple exposures to more easily obtain publication-quality blot images. This reaction produces a prolonged chemiluminescence which can be visualized on X-ray film or an imaging system.

Ordering Information:

Cat No.	Components	E665966-50 mL	E665966-250 mL
E665966A	eECL-A (Luminol Enhancer)	25 mL	125 mL
E665966B	eECL-B (Peroxide)	25 mL	125 mL

Protocol:

1. Wash the membrane 6 times for 5 minutes each in wash buffer to remove any unbound secondary antibody conjugate. It is crucial to thoroughly wash the membrane after incubation with the HRP enzyme conjugate.
2. Prepare the substrate working solution by mixing equal parts of the eECL-A and eECL-B components (e.g. 1mL substrate working solution for a 6cm × 8cm membranes). Use a sufficient volume to ensure that the blot is completely wetted with the substrate and the blot does not become dry.
3. Incubate the membrane with the substrate working solution for 3-5 minutes.
4. Remove blot from working solution and place it in a plastic sheet protector or clear plastic wrap.
5. Use an absorbent tissue to remove excess liquid and carefully press out any bubbles from between the blot and the membrane protector.
6. Image the blot using an imaging system or X-ray film.

Troubleshooting:

Observation	Cause	Solution
Reverse image on film (white bands with a black background)	Too much HRP in the system	Further dilute the HRP-conjugate
Membrane has brown or yellow bands		
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	Further dilute the HRP-conjugate
	Insufficient quantities of antigen or antibody	Increase amount of antibody or antigen
	Reduction of HRP or substrate activity	Optimize transfer conditions
High background	Too much HRP in the system	Further dilute the 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
Spots within the protein bands	Inefficient protein transfer	Optimize transfer conditions
	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 0.2 μm filter
Nonspecific bands	Too much 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
	Insufficient blocking	Increase blocking time or use different blocking reagent