MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

eECL Western Blot Kit

Product Number: WB049

Shipping and Storage

2-8 ℃, stored in dark

Components

Component	WB049 50ml	WB049 250ml
eECL-A(Luminol Enhancer)	25 ml	125 ml
eECL-B(Peroxide)	25 ml	125 ml

Description

The eECL Western Blot Kit is a highly sensitive and enhanced detection kit used in immunoblotting experiments in conjunction with horseradish peroxidase (HRP). This product is developed based on a new generation of enhanced chemiluminescent substrates, which undergo chemical reactions under the catalysis of HRP and emit light. It can be used to detect biomolecules such as proteins fixed on membranes. Its high sensitivity can detect PG level antigens, and the luminescent signal is strong and persistent. It can be detected using X-ray film exposure or chemiluminescence imaging equipment.

Note

1. During contact with the membrane, please wear gloves and use clean equipment such as tweezers to avoid protein contamination and high background.
2. Under dark conditions, the prepared chemiluminescence detection substrate working solution can be stably stored at room temperature for 8 hours. Sunshine or other strong light can affect the working fluid, so prolonged exposure to strong light should be avoided. Short term exposure to normal laboratory lighting does not affect the use of working fluids.
3. Our company provides a variety of protein transfer membranes, blocking solutions, primary antibodies, enzyme-linked secondary antibodies, buffer solutions, etc. Please refer to our company website for details.

Protocol

1. After the second antibody incubation is completed, wash the imprinting film thoroughly.
2. According to the required amount, mix eECL-A and eECL-B in a 1:1 ratio and equal volume to prepare a chemiluminescence detection substrate working solution (approximately 1 ml of working solution is used for an 8 cm x 6 cm membrane).
3. Discard the washing buffer and drop the luminescent substrate working solution onto the imprinting film, ensuring that the working solution covers the entire film. Incubate at room temperature for 3-5 minutes.
4. Use a pipette to remove excess luminescent substrate working solution and place the imprint film between two clean plastic films. This process should be completed carefully to avoid the formation of bubbles between films.
5. Expose X-ray film in a darkroom or place the film in a chemiluminescence imager and perform testing according to the instrument manual.

Schedule

Problem	Reason	Resolution
Film inversion (White stripe, black background)	Excessive HRP in the system	Dilute HRP markers at least 10 times or more
Brown or yellow stripes appear on the membrane		

Strong luminescence seen in the darkroom		
The duration of the luminous signal is too short		
Weak or no signal	Excessive HRP in the luminescent reaction system leads to rapid substrate consumption, resulting in rapid signal reduction	Dilute HRP markers at least 10 times
	Insufficient antigen/antibody levels	Increase antigen/antibody usage
	Low protein transfer rate	Optimize transfer system
High background	Excessive HRP in the system	Dilute HRP markers at least 10 times
	Insufficient closure	Optimize closed programs
	Improper selection of sealing reagents	Choose another blocking reagent
	Insufficient flushing	Increase flushing time and frequency
	Overexposure	Reduce exposure time
	Antigen/antibody concentration too high	Reduce antigen/antibody usage concentration
The protein bands are punctate	Protein transfer failure	Optimize the transfer process
	Membrane imbalance	Handle the film according to the instructions
	There are bubbles between the film and film	Remove all bubbles before exposure
Non specific bands appear (High background, short signal maintenance time)	There are too many HRPs in the system	Dilute HRP markers
Non specific bands appear (The background is clean and the signal maintenance time is normal)	Excessive dosage of primary antibody	Further dilution of primary antibody
	SDS leads to non-specific binding	Avoid using SDS during the experiment