Super ECL Kit

(Ultrasensitive Chemiluminescence Detection Kit)

Cat. No./Spec.

D1001/10 ml, D1002/100 ml, D1003/500 ml

Component

Components	D1001	D1002	D1003
A Solution	5 ml	50 ml	250 ml
B Solution	5 ml	50 ml	250 ml

Storage Condition

Store at 4°C, sealed, and protected from light. For short-term storage, it can be kept at room temperature.

Product Description

The Super ECL Kit is used for detecting antibodies labeled with horseradish peroxidase (HRP) in Western Blotting experiments. This product's immunoblotting substrate is an enhanced chemiluminescence (ECL) HRP substrate that enables the detection of low-expressed or high-value proteins in immunoblotting analysis. This ECL substrate is compatible with various membranes, blocking solutions, and a wide range of antibody dilutions.

Product Features

1. Extremely high sensitivity: Picogram-level sensitivity;
2. Strong compatibility: Can detect protein bands on nitrocellulose or PVDF membranes;
3. Long signal duration: Under optimized conditions, the blot bands incubated with the substrate can emit a detectable light signal for 6 to 8 hours;
4. High stability: The working solution remains stable for up to 24 hours;
5. Economical pricing: The formulation is optimized for the detection of antibodies at very low concentrations
   - 0.2-1.0 μg/mL primary antibody (or 1 mg/mL dilution range 1:1,000-1:5,000)
   - 10-50 ng/mL secondary antibody (or 1 mg/mL dilution range 1:20,000-1:100,000)

Usage Method

1. Perform routine SDS-PAGE electrophoresis, membrane transfer, and Western blotting steps. Incubate with 0.2-1.0 μg/mL primary antibody for 1 hour or overnight, then wash the membrane and incubate with 10-50 ng/mL secondary antibody for 30-60 minutes.
2. Prepare the luminescent working solution during the last wash of Western blotting: Take equal volumes of Solution A and Solution B, mix in a clean container, and let it stand at room temperature for later use. Note: It is recommended to use the working solution immediately; sensitivity may slightly decrease if left at room temperature for several hours.
3. Take the membrane out with tweezers, drain the wash solution on filter paper but do not let the membrane dry completely. Immerse the membrane completely in the luminescent working solution (0.1 mL luminescent working solution/cm^2 of membrane), ensuring full contact with the working solution. Incubate at room temperature for 3-5 minutes.
4. Take the membrane out, discard the luminescent working solution, and slightly absorb the excess liquid with absorbent paper. Place the membrane between two sheets of plastic wrap, then proceed with film pressing detection or imaging detector detection.
5. Detection
   - Film pressing detection: Fix the membrane in a film clip with the protein band facing up. Press in the darkroom for 1 minute, then develop and fix immediately. Adjust the pressing time based on the results, or press for 30 seconds, 1 minute, 3 minutes, and 5 minutes separately, then develop and fix together to observe the results.

- Imaging detector detection: Place the membrane in the imaging detector and follow the instrument manual for detection.

Important Notes

1. Steps 1 to 4 can be performed under fluorescent lights, but the sensitivity of the luminescent liquid may slightly decrease if exposed to strong light for too long. Moving to a darkroom can avoid this. Wearing gloves can prevent leaving fingerprints on the membrane.
2. Long exposure or excess protein will deepen the background and cause the band strength to lose linearity, while insufficient exposure will make the bands blurry.
3. After about 3 minutes of incubation with the luminescent working solution, the bands on the membrane will glow. Strong bands are visible to the naked eye in the darkroom, while low-abundance protein bands may be too weak to see but can expose X-ray film. Do not judge the band glow time simply by naked eye observation. Invisible fluorescence can last for several hours and expose X-ray film, so weak bands can be exposed for 1-10 hours. If the bands are not satisfactory after exposure, the membrane can be washed with wash buffer, re-incubated with the secondary antibody, and then re-exposed with ECL luminescence.

Common Problems and Solutions

Problem	Possible Cause	Solution
Reverse image (white bands on a black background)	Excessive HRP in the system	Further dilute the HRP conjugate
Brown or yellow bands on the membrane		
Dirt in the darkroom		
Signal duration less than 8 hours		
Signal is weak or nonexistent	An excess of HRP in the system can deplete the substrate, leading to a rapid decline in the signal.	Further dilute the HRP conjugate
	Insufficient antigen or antibody	Increase the amount of antigen or antibody
	Low transfer efficiency	Optimize transfer
High background	Excessive HRP in the system	Further dilute the HRP conjugate
	Inadequate blocking	Optimize blocking conditions
	Inappropriate blocking solution	Try a different blocking solution
	Insufficient washing	Increase wash time, number of washes, or wash solution volume
	Overexposure	Reduce exposure time
	Antigen or antibody concentration too high	Lower antigen or antibody concentration
Spots within the protein bands	Low transfer efficiency	Optimize transfer procedures
	Uneven hydration of the membrane	Follow the manufacturer's recommended hydration process
	Air bubbles between membranes	Remove bubbles before exposure

This product is for research use only.