ECL Western Blotting Detection Reagents

Cat. Number: ECL

Storage: 2-4 °C. Don't freeze. Protect from light.

Description:

Cyrusbioscience ECL Western Blotting Detection Reagents is a ready-to-use reagent for chemiluminescent detection of immobilized proteins (Western blotting), conjugated with horse -radish peroxidase (HRP) directly or indirectly. In the presence of hydrogen peroxide, HRP catalyzes the oxidation of cyclic diacylhydrazides, such as luminol, and light emits.

Cyrusbioscience ECL Western Blotting Detection Reagents provides a convenient way to visualize HRP-based detection. Simply mix and add the solutions onto the membrane. The signal of target proteins can be recorded by exposure to X-ray films or compatible image acquisition system.

Duration: >1Hr	**Detection Method:** X-ray film or imaging acquisition system
Typical Antibody dilution:	Primary: 1:1,000–1:20,000, Secondary: 1:20,000–1:200,000
Shelf life: >1 year at time of receipt	**Recommended Initial Exposure Time:** 3 minutes (X ray film)

Components:

ECL Western Blotting Detection Reagents A	250mL	1 bottle
ECL Western Blotting Detection Reagents B	250mL	1 bottle

Safety Information:

Please wear gloves, lab coat and goggles while operating. Prevent contact product directly. In case of contacting, wash with large amount of water.

Storage: Stored at 4°C and shielded from light. Please use up the product in 1 year.

Materials needed but not provided:

1. PVDF or nitrocellulose membrane.
2. Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05–0.1% Tween-20
   PBS: 10 mM sodium phosphate, 150 mM NaCl, pH 7.2
   TBS: 10 mM Tris, 150 mM NaCl, pH 7.4
3. Blocking buffer: 1–5% (w/v) blocking agent (e.g., casein, BSA, or gelatin) in wash buffer
4. Specific primary antibody for interested protein, diluted in blocking buffer
5. HRP-conjugated secondary antibody, specific for primary antibody, diluted in blocking buffer
6. X-ray film or chemiluminescence image acquisition systems

Instruction:

A. Protein transfer

1. A Perform 1D or 2D electrophoresis for protein separation.
2. Move the electrophoretic gel into appropriate transfer buffer and equilibrate for 10 minutes.
3. Wet the PVDF or nitrocellulose membrane in transfer buffer. (For PVDF membrane, it is necessary to pre-wet it in methanol before moving into transfer buffer).
4. Assemble the transferring sandwich as the order of two filter papers, gel, membrane and two filter papers.
5. Transfer proteins according to blotting apparatus manufacturer's instruction.

B. Antibody incubation

1. Add BSA, skim milk based blocking buffer and incubate at room temperature for 30 minutes.
2. Prepare the primary antibody by diluting it with blocking buffer according to the manufacturer's instruction or previous experience.

NOTE: *Due to the good sensitivity of chemiluminenscent detection, previous antibody dilution factor can be increased 2-5 folds for optimal signal to noise ratio.*

3. Add primary antibody and incubate at room temperature for at least 1 hour with gentle agitation. For more specific interaction between primary and antigen proteins, it is recommended to perform additional incubation at 4°C for 8-12 hours.
4. Decant the primary antibody solution thoroughly. Wash the membrane of at least three times with ample amount of fresh Wash buffer for 10 minutes.
5. Prepare the secondary antibody by diluting it with blocking buffer according to the manufacturer's instruction.

NOTE: Due to the good sensitivity of chemiluminenscent detection, secondary antibody dilution can start from 1:20,000.

6. Add secondary antibody and incubate at room temperature for 1 hour with gentle agitation.
7. Decant the secondary antibody solution thoroughly. Wash the membrane of at least four times with ample amount of fresh Wash buffer for 10 minutes.

C. Chemiluminenscent detection

1. To prepare working HRP substrate, mix equal volume of Solution A and Solution B in a clean tube freshly. 0.1 mL of working HRP substrate is sufficient per 1 cm^2 membrane area.

NOTE: The HRP working substrate stands at room temperature for 3 minutes with proper light shielding, it can provide more stable signal.

2. In the dark room or box, place the membrane side up in a clean box or plastic wrap. Add HRP working substrate onto the membrane.
3. Incubate the membrane at room temperature for 1 minute.
4. Drain excess HRP substrate
5. Expose the membrane to appropriate X-ray film or by chemiluminenscent image acquisition system. It is

recommended to use 3 minutes as the initial exposure time.

D. Stripping of PVDF membrane

The immunoblot of PVDF membrane can be stripped of antibodies, and then reprobed.

1. Incubate membrane in stripping buffer for 30 minutes at 50-70 OC.
2. Wash the membrane twice in Wash buffer for 10 minutes each.
3. To ensure complete removal of antibodies, incubate the membrane with CyECL Western Blotting working substrate and expose against X-ray film for 5 minutes. No signal should be observed for complete stripping.

Trouble Shooting:

Problem	Possible cause	Remedy
No signal or weak signal	Poor transfer efficiency	Optimize the membrane transferring procedure.
	Insufficient antigen	Increase the amount of loaded antigen. Make sure the blot have been store correctly to avoid the degradation of target protein.
	The concentration of primary and secondary antibody is too low	Increase the concentration of the primary and/or the secondary antibody.
	Inappropriate storage/preparation of the ECL detection reagents	Use HRP or HRP conjugates to check the applicability of ECL reagents.
	Too short exposure time	Extend exposure time
Excessive signal	Antigen or antibody excess	Reduce the amount of loaded antigen. Dilute the primary antibody and/or the secondary antibody.
High Background	Antigen or antibody excess	Optimize the condition by reducing the amount of antigen, or the concentration of the primary antibody and/or secondary antibody. Initially, reduce the secondary antibody to 20% of the original usage.
	Inappropriate blocking	Try different blocking substrate such as BSA, gelatin, casein.
	Inadequate washing	Increase the concentration of Tween-20 in washing solution. Increase the washing steps between the hybridization procedures. Extend washing time.
	Overexposure to film	Shorten the exposure time.

Note: 如以 X-ray Film 壓片方式偵測，在壓片前，請勿吸乾轉印膜上殘存試劑，使轉印膜保持濕潤情況下進行壓片。

建議在轉印膜上可使用乾淨投影片，再放 X-ray Film 進行壓片。如吸乾轉印膜上試劑，將會使冷光效果大幅下降。