

ClearBand ECL Western Blotting Substrate

Troubleshooting Guide

50 ml, 200 ml

Cat No: ECL50, ECL200

Use this guide for common chemiluminescent detection problems with ClearBand ECL Western Blotting Substrate. The substrate is a luminol-based system for HRP detection on immunoblots and is used as a freshly prepared 1:1 mixture of Solution A and Solution B.

Before You Start - Quick Checks

1. Store the substrate at 4°C.
2. Prepare Solution A and Solution B at 1:1 immediately before use.
3. Use approximately 0.125 mL working solution per cm² of membrane.
4. Incubate the membrane for about 3 minutes, remove excess reagent, eliminate bubbles under the clear wrap, and expose promptly.

Problem	Most likely cause	Recommended action
No chemiluminescent signal	The detection system may not be HRP-based or the working solution may not have been prepared correctly.	Confirm HRP detection and prepare fresh Solution A + Solution B at a 1:1 ratio immediately before use.
Signal is weak	Too little working solution may have been used or membrane contact may be incomplete.	Use about 0.125 mL/cm ² and ensure the entire membrane is evenly covered during the approximately 3-minute incubation.
Signal is uneven across the blot	The membrane may not have been uniformly exposed to substrate or bubbles may remain under the protective wrap.	Cover the entire blot with working solution, drain excess, then remove bubbles completely before imaging.
Some areas show no signal	Air pockets may have prevented substrate or imaging-film contact.	Reapply the workflow with uniform substrate coverage and smooth the clear wrap or sheet protector to eliminate bubbles.
Working solution gives inconsistent results	The A/B mixture may have been prepared too early or reused.	Prepare a fresh 1:1 working solution immediately before each use and do not store mixed working reagent for later runs.
Background is unexpectedly variable	Excess reagent, uneven draining, or uneven membrane contact may vary between runs.	Use the specified working-solution volume, drain excess consistently, and wrap the membrane without trapped bubbles.
Image exposure is inconsistent between replicates	Timing between the 3-minute substrate incubation and exposure may differ.	Standardize the incubation, draining, wrapping, and exposure sequence across membranes.
Substrate performance changed after storage	Storage may not have followed the datasheet condition.	Return the stock components to 4°C storage and use fresh working solution for the next blot.

Still having trouble? Confirm HRP-based detection, 4°C storage, a fresh 1:1 A/B mixture, 0.125 mL/cm² coverage, the 3-minute incubation, and bubble-free wrapping before repeating exposure.