

ClearBand Super ECL Western Blotting Substrate

Troubleshooting Guide

20 mL, 50 mL

Cat No: SECL20, SECL50

Use this guide for common chemiluminescent detection problems with ClearBand Super ECL Western Blotting Substrate. The femto-level HRP substrate is supplied as two solutions that are mixed 1:1 immediately before use and applied at 0.1 mL working solution per cm² of membrane.

Before You Start - Quick Checks

1. Prepare a fresh working solution immediately before use by mixing Solution A and Solution B at 1:1.
2. Use approximately 0.1 mL working solution per cm² of membrane and incubate for about 2 minutes at room temperature.
3. Drain excess reagent, cover the blot without bubbles, and then expose using film or an imaging system.

Problem	Most likely cause	Recommended action
No chemiluminescent signal	The A:B mixture may be incorrect, the working solution may not be fresh, or HRP may be absent/inactive.	Prepare fresh Solution A + B at 1:1 immediately before use and confirm that an HRP-conjugated detection system is present and functional.
Very weak signal	Too little working solution, insufficient membrane contact, or an underexposed image may reduce sensitivity.	Use 0.1 mL/cm ² , ensure complete membrane coverage for about 2 minutes, drain excess reagent, and optimize imaging exposure.
Very high background	The blot may contain excess HRP-labeled antibody, insufficient washes, or the imaging exposure may be too long.	Improve post-antibody washing and reduce exposure time; if needed, optimize antibody concentrations before changing the ECL formulation.
Signal is saturated	The femto-level substrate can produce very strong signal when target abundance or HRP level is high.	Shorten exposure and/or reduce antibody concentration in the immunoblot workflow.
Uneven or patchy signal	The membrane may not have been uniformly covered with working solution or bubbles may be trapped during imaging.	Apply sufficient reagent to wet the entire membrane uniformly and remove bubbles before exposure.
Signal fades rapidly	The working solution may have been prepared too early or imaging may have been delayed.	Mix Solutions A and B immediately before use and proceed promptly through the 2-minute incubation, draining, wrapping, and imaging steps.
Background appears only in some regions	Residual wash buffer, local reagent pooling, or uneven membrane contact may create regional differences.	Drain the blot consistently, distribute working solution uniformly, and place the membrane flat without trapped bubbles.
Reproducibility differs between blots	A:B ratio, reagent volume per area, incubation time, or exposure settings may vary.	Standardize the 1:1 mix, 0.1 mL/cm ² volume, ~2-minute room-temperature incubation, and imaging exposure across experiments.

Still having trouble? Recheck a fresh 1:1 A:B mixture, 0.1 mL/cm² coverage, the ~2-minute incubation, bubble-free wrapping, antibody washing, and exposure settings before changing the immunoblot reagents.