

# Clarity™ and Clarity Max™ Western ECL Substrates

## Quick Start Guide



Catalog #	Description
1705060	<b>Clarity Western ECL Substrate</b> , 200 ml
1705061	<b>Clarity Western ECL Substrate</b> , 500 ml
1705062	<b>Clarity Max Western ECL Substrate</b> , 100 ml

**BIO-RAD**

## Introduction

Bio-Rad's Clarity family of substrates are compatible with any HRP-conjugated secondary antibody and are ideal for both digital and film-based imaging.

The **Clarity ECL Substrate** provides excellent sensitivity, very low background levels, and an extremely long signal duration. This produces exceptionally clear images and allows re-imaging without loss of signal.

The **Clarity Max ECL Substrate** provides the highest level of sensitivity while maintaining low background and is recommended for detection of low target amounts.

## Storage Conditions

Clarity and Clarity Max ECL Substrates are stable at room temperature.

## Quick Start Protocol

1. After immunodetection, keep the membrane moist in wash buffer as you prepare the substrate mixture.
2. Mix substrate kit components in a 1:1 ratio. Prepare 0.1 ml of solution/cm<sup>2</sup> of membrane.
  - For a mini-sized membrane (7 x 8.5 cm), 7 ml of solution is sufficient
  - For a midi-sized membrane (8.5 x 13.5 cm), 12 ml of solution is sufficient
3. Incubate the membrane in the substrate solution for 5 min.
4. Image the membrane with a digital imager or by exposing to X-ray film.

## Optimization Tips

Problem	Solution
High background	<ul style="list-style-type: none"><li>▪ Increase the concentration of blocking agent</li><li>▪ Increase the number or stringency of washes</li><li>▪ Decrease antibody concentrations</li></ul>
No reaction or weak signal	<ul style="list-style-type: none"><li>▪ Increase antibody concentration or incubation times</li><li>▪ Remove excess wash buffer from the membrane to ensure peroxidase is at optimal pH</li><li>▪ Sodium azide inhibits horseradish peroxidase. Use a different preservative for antibody stocks</li></ul>
Areas of no signal within a band (a donut appearance)	<ul style="list-style-type: none"><li>▪ Load less sample or use lower concentrations of primary or secondary antibody to avoid localized substrate exhaustion</li></ul>
Blank spots on membrane	<ul style="list-style-type: none"><li>▪ Ensure that membranes are not allowed to dry during immunodetection</li><li>▪ Ensure that substrate completely and evenly covers membrane</li></ul>

Refer to the full instruction manual available at [bio-rad.com/web/ClarityManuals](http://bio-rad.com/web/ClarityManuals) for more information.

Clarity Max Western ECL Substrate is manufactured by Cyanagen Srl and is the subject of patent application numbers US7855287, EP1950207, US9040252, AU2011202658, CA2742025, US8129136, and EP1962095, together with other equivalent granted patents and patent applications in other countries like CN102313732.