Clarity and Clarity Max Western ECL Substrates

Instruction Manual

Catalog #1705060	Clarity Western ECL Substrate, 200 ml
1705061	Clarity Western ECL Substrate, 500 ml
1705062	Clarity Max Western ECL Substrate, 100 ml

For technical support, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.
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Section 1
Introduction

Clarity and Clarity Max Western ECL Substrates are compatible with any horseradish peroxidase (HRP)–conjugated secondary antibody and are ideal for both digital and film-based imaging. Clarity and Clarity Max Substrates provide excellent sensitivity with a long signal duration that allows reimaging without loss of signal. In addition, Clarity and Clarity Max Substrates are formulated to exhibit very low background levels that yield exceptionally clear images. The combination of bright, long signal and low background makes the Clarity family of substrates the perfect choice for most blotting applications.

Section 2
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^2 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. Place the membrane protein side up on a clear surface.
   – Add substrate to the blot and incubate for 5 min
4. Image the membrane with a digital imager or by exposing to X-ray film

Section 3
Storage Conditions

Clarity and Clarity Max Substrates are stable at room temperature.

Section 4
Background on Chemiluminescence

Chemiluminescence is a chemical reaction that produces light and has become a common detection method for western blotting because of its high sensitivity. With chemiluminescent western blots, a secondary antibody is conjugated to HRP. Once the secondary reagent is bound to the target protein on the membrane, the membrane is incubated with a solution containing the chemiluminescent substrate (Figure 1). In the presence of peroxide, HRP catalyzes the oxidation of luminol, which then generates light (Figure 2). An enhancer is included in the substrate solution to increase the longevity and intensity of the emitted light. Depending on the substrate and enhancer formulation, the half-life of the light-generating reaction can range from a few minutes to more than an hour. The light resulting from this reaction can be detected with either film or a digital imaging system. When used with the Bio-Rad™ ChemiDoc or ChemiDoc MP Imaging System, Clarity and Clarity Max Substrates produce clear digital images that can be directly used for analysis or publication.

Section 5
Antibody Incubation

A typical immunodetection experiment system utilizes two sets of antibodies.

- The primary antibody, which is directed against the target protein (antigen)
- The secondary reagent, in this case an antibody that recognizes and binds to the primary antibody; it is conjugated to an enzyme such as HRP, which will convert the substrate into light, which is then detected by a film imager

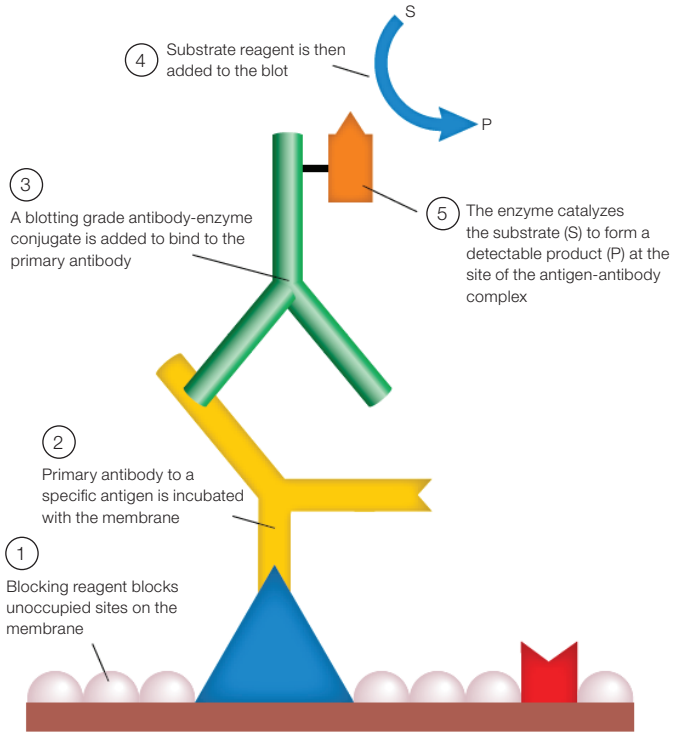

Fig. 1. Specific enzymatic detection of membrane-bound antigens.

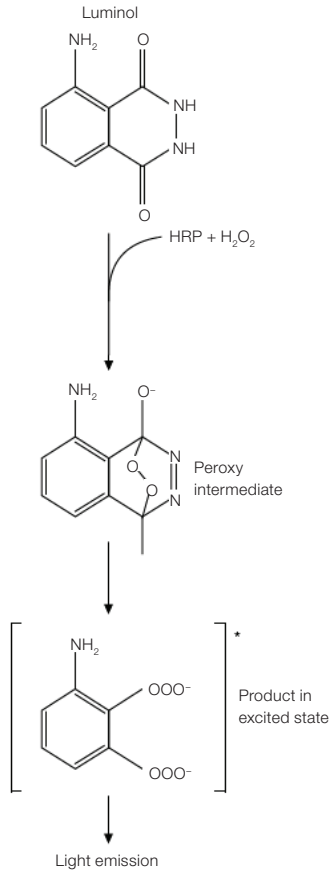

Fig. 2. Chemiluminescence detection by HRP. The secondary antibody is linked to an enzyme, which catalyzes a reaction leading to light emission. Luminol oxidized by HRP in the presence of a peroxide leads to the formulation of a 3-aminophthalate dianion and the release of light.

Section 6
Detailed Assay Procedure

Example Western Protocol

Materials

- Low fluorescence polyvinylidene difluoride (LF PVDF), PVDF, or nitrocellulose membrane with transferred proteins
- Blocking buffer (Tris buffered saline [TBS] or phosphate buffered saline [PBS]) with 0.05% Tween-20 and 1–6% blocking reagent, typically bovine serum albumin (BSA), gelatin, casein, or nonfat dry milk
- Wash buffer (TBS or PBS with 0.05% Tween-20)
- Primary antibody, diluted in blocking buffer
- HRP-conjugated secondary reagent, such as goat anti-rabbit or goat anti-mouse conjugated HRP, diluted in wash buffer

Immunodetection

1. Wash buffer volumes should be at least 20 ml for mini blots and 100 ml for midi blots. Block and antibody solution volumes should be at least 10 ml for mini blots and 25 ml for midi blots.
2. Equilibrate membrane with transferred proteins in wash buffer for 3 min. Dried LF PVDF and PVDF membranes should be briefly rewet in methanol prior to equilibration in wash buffer.
3. Incubate the membrane, protein side up, in blocking buffer for 1 hr with continuous agitation.
4. Incubate the membrane in diluted primary antibody solution for 1 hr with continuous agitation.

   – Incubation in primary antibody may be carried out overnight at 4°C
5. Wash the blot in wash buffer five times for 5 min each with continuous agitation.
6. Incubate the blot in diluted secondary antibody solution for 1 hr with continuous agitation.
7. Wash the blot in wash buffer six times for 5 min each with continuous agitation.

Chemiluminescent Development

1. Keep the membrane moist in wash buffer as you prepare the substrate mixture.

   – Do not allow the membrane to dry out during the subsequent steps
2. Mix substrate kit components in a 1:1 ratio. Prepare 0.1 ml of solution/cm^2 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. Place the membrane, protein side up, on a clear surface.
   – Add substrate to the blot and incubate for 5 min
   – Ensure the surface of the blot is completely covered with substrate with no air bubbles
4. Remove the membrane from the substrate solution and drain off excess.
5. Place membrane in a plastic sheet protector or in plastic wrap to prevent the membrane from drying.
6. Image the membrane with a digital imager or by exposing to X-ray film.
   – If switching to Bio-Rad Clarity or Clarity Max from another substrate, optimal exposure times may be different

Section 7
Troubleshooting

Problem	Cause	Solution
High background	Blocking was incomplete	■ Increase the concentration of blocking agent or increase blocking duration ■ Match the blocker to the membrane. For example, gelatin may give poor results on PVDF membranes
	Washing was insufficient	■ Increase the number, duration, or stringency of the washes
	The primary or secondary antibody was too concentrated	■ Decrease antibody concentrations ■ Perform a dot blot experiment to optimize the working concentrations
Areas of no signal within a band (a donut appearance)	Localized substrate depletion	■ Bands on the blot with high protein amounts will lead to excessive local concentrations of peroxidase conjugate. This may lead to localized substrate depletion. Use lower concentrations of primary and secondary antibody ■ Load less sample
No reaction or weak signal	Proteins may be washed from the membrane during assays	■ Reduce the number or stringency of washes
	Antigen binding to the membrane was insufficient	■ Decrease antibody concentrations ■ Stain the gel after transfer or use prestained standards to assess transfer efficiency ■ Some total protein stains (such as amido black and colloidal gold) interfere with antibody recognition of the antigen. We recommend use of other total protein stains ■ Optimize the blocking reagent. Some blocking reagents (such as nonfat dry milk) provide high stringency at the expense of sensitivity. Others (such as BSA) offer comparatively high sensitivity at the expense of higher background or nonspecific binding
	Poor antibody binding to the antigen	■ Detergents may affect the binding of some antibodies. Eliminate or reduce their amount in the assay ■ Increase the antibody incubation times
	Insufficient reagent volume	■ Use additional volumes of blocking, antibody, and wash solutions
	The enzyme conjugate was inactive	■ Test the reagent for activity ■ HRP is most active at optimal pH. Ensure excess wash buffer is removed from the membrane before application of substrate ■ Sodium azide is a potent inhibitor of HRP. Eliminate from antibody stocks
Blank spots in areas of membrane that should have signal	The membrane was allowed to dry during handling	■ Ensure that no air bubbles are present during assembly of transfer stack ■ Ensure that warm membranes are not allowed to dry after transfer

Section 8
Ordering Information

Catalog #	Description
1705060	**Clarity Western ECL Substrate**, 200 ml, contains 100 ml Clarity Western Peroxide Reagent and 100 ml Clarity Western Luminol/Enhancer Reagent
1705061	**Clarity Western ECL Substrate**, 500 ml, contains 250 ml Clarity Western Peroxide Reagent and 250 ml Clarity Western Luminol/Enhancer Reagent
1705062	**Clarity Max Western ECL Substrate**, 100 ml, contains 50 ml Clarity Max Western Peroxide Reagent and 50 ml Clarity Max Western Luminol/Enhancer Reagent

Visit **bio-rad.com/ClaritySubstrates** 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.

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