Luminata™ Crescendo ELISA HRP Substrate

Introduction

Luminata Crescendo ELISA HRP Substrate is a chemiluminescent reagent designed for ELISA applications that use luminometry to detect horseradish peroxidase (HRP)-conjugated labels. It is a two-component system consisting of a luminol reagent and a peroxide solution. A working solution is prepared by combining equal volumes of the luminol and peroxide solutions. The working solution is stable for approximately 2 hours and can detect IL-2 protein (as determined in an IL-2 sandwich ELISA) down to 0.4 picograms (pg).

Package Contents

Luminata Crescendo ELISA HRP Substrate 50 mL of Luminol Reagent 50 mL of Peroxide Solution Catalogue number ELLUR0100	*volume adequate for 10 x 96-well plate assays*
Luminata Crescendo ELISA HRP Substrate 100 mL of Luminol Reagent 100 mL of Peroxide Solution Catalogue number ELLUR0200	*volume adequate for 20 x 96-well plate assays*

Storage/Shelf Life

Store at 2–8 °C. Refer to bottle label for expiration date.

Usage Guidelines

- Prior to using this product, review the Material Safety Data Sheet (MSDS) to ensure awareness of associated hazards and use of appropriate controls.
- Prepare a working solution of Luminata Crescendo ELISA Substrate by combining equal volumes of Luminol Reagent and Peroxide Solution. Store working solution in a dark container.
- Do not use sodium azide in blocking buffers or wash solutions, since it inhibits HRP activity.
- Optimization of antibody, antigen, and/or HRP-conjugated antibody concentrations is required. High HRP concentrations can lead to rapid substrate consumption and rapid signal decay.
- Use of Tween® 20 surfactant (up to 0.05%) in the blocking solution may reduce background.

Sandwich ELISA Example

One of the most common formats for the ELISA assay is the sandwich ELISA, where the analyte is bound between the capture antibody and the detection antibody. The antibodies can be monoclonal or polyclonal but they must recognize two non-overlapping epitopes. The following protocol is a general example of an IL-2 sandwich ELISA in a 96-well plate. Optimization of the specific antibody/antigen system is required.

1. Coat the wells of a high-binding 96-well plate in 100 µL of capture antibody (diluted to 1 µg/mL in an appropriate buffer) and incubate at 4 °C overnight.
2. Wash the plate 3 times with 100 µL of Tris Buffer Saline containing 0.05% Tween 20 surfactant (TBS-T) to remove the unbound antibody. Agitate the plate during washes to reduce nonspecific binding.
3. Block the unoccupied sites for 1 hour by adding 100 µL of a suitable blocking solution containing a surfactant (e.g. 1% BSA in TBS-T or bløk™-CH Buffer). Agitate during blocking.
4. Add 100 µL of antigen and/or IL-2 standard and incubate for 2 hours with agitation.
5. Wash the plate 3 times with TBS-T as in step 2.
6. Incubate with 100 µL of detection antibody (0.1 µg/mL) for 1 hour with agitation.
7. Wash the plate 3 times with TBS-T as in step 2.
8. Incubate with 100 µL of HRP-conjugated secondary antibody (0.1 µg/mL) for 1 hour with agitation.
   NOTE: Secondary antibody must **not** recognize capture antibody.
9. Wash 3 times with TBS-T as in step 2.
10. Add 100 µL of Luminata Crescendo ELISA Substrate working solution and incubate for 5 minutes with agitation.
11. Immediately read the 96-well plate in a luminometer.

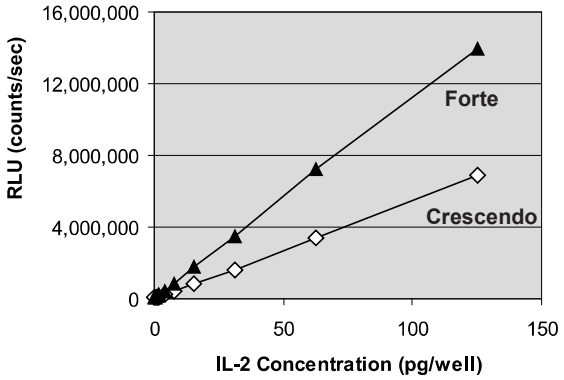
Indirect ELISA Example

The indirect ELISA assay is used primarily to determine the amount of a specific antibody in a sample (e.g., serum from an immunized animal or hybridoma supernatant). This method is sensitive and versatile, since the primary antibody can bind several epitopes on the antigen, and the labeled secondary antibody can bind multiple sites on the primary antibody, allowing for signal amplification. The following protocol is a general example of an indirect ELISA. Optimization of the specific antibody/antigen system is required.

1. Add 100 µL of antigen-containing sample or standard, diluted in an appropriate buffer, to the wells of a high-binding 96-well plate and incubate at 4 °C overnight.
2. Wash the plate 3 times with 100 µL of Tris Buffer Saline containing 0.05% Tween 20 surfactant (TBS-T) to remove the unbound antigen. Agitate the plate during washes to reduce nonspecific binding.
3. Block the unoccupied sites for 1 hour by adding 100 µL of a suitable blocking solution containing Tween 20 surfactant (e.g. 1% BSA in TBS-T or bløk-CH Buffer). Agitate during blocking.
4. Incubate with 100 µL of primary antibody (0.1–1.0 µg/mL) for 1 hour with agitation.
5. Wash the plate 3 times with TBS-T as in step 2.
6. Incubate with 100 µL of HRP-conjugated secondary antibody (0.1 µg/mL) for 1 hour with agitation.
7. Wash 3 times with TBS-T as in step 2.
8. Add 100 µL of Luminata Crescendo ELISA Substrate working solution and incubate for 5 minutes with agitation.
9. Immediately read the 96-well plate in a luminometer.

Proof of Performance

Standard Curves for Luminata Crescendo and Forte ELISA Substrates



Substrate sensitivity was determined using IL-2 sandwich ELISA. Recombinant mouse IL-2 was serially diluted in a white 96-well plate and detected using IL-2 ELISPOT antibody pair (Millipore cat. no. ELI-002-M). Luminescence was measured using a Wallac VICTOR²™ 1420 multilabel counter.

Troubleshooting

Symptom	Possible Cause	Solution
High background	High HRP-conjugate concentration Incomplete washing	Decrease HRP-conjugate concentration. Wash plate wells thoroughly. Blot 96-well plate on paper towels to empty wells. Change the blocking solution.
Signal disappears quickly	High HRP-conjugate concentration has exhausted the substrate prematurely	Decrease HRP-conjugate concentration. Decrease antigen concentration.
Weak or no signal	Ab concentration is too low	Use a higher sensitivity HRP detection substrate. Increase Ab concentration. Increase antigen concentration.

Ordering Information

This section lists the catalogue numbers for Luminata Crescendo ELISA Substrate and related products. See the Technical Assistance section for information about contacting Millipore. You can also purchase Millipore products on-line at www.millipore.com/products.

Luminata and Immobilon® Western Chemiluminescent Substrates for ELISA and Western Blotting Applications

Description	Qty	Cat. No.
Luminata Crescendo ELISA HRP Substrate	100 mL	ELLUR0100
	200 mL	ELLUR0200
Luminata Forte ELISA HRP Substrate	100 mL	ELLUF0100
Luminata Classico Western HRP Substrate	100 mL	WBLUC0100
	500 mL	WBLUC0500
Luminata Crescendo Western HRP Substrate	100 mL	WBLUR0100
	500 mL	WBLUR0500
Luminata Forte Western HRP Substrate	100 mL	WBLUF0100
	500 mL	WBLUF0500
Immobilon Western Chemiluminescent HRP Substrate	50 mL	WBKLS0050
	100 mL	WBKLS0100
	500 mL	WBKLS0500

Ordering Information, continued

SNAP i.d.® Protein Detection System and Components

Description	Qty	Cat. No.
SNAP i.d. Protein Detection System	1	WBAVDBASE
SNAP i.d. Triple Well Blot Holder	20	WBAVDBH03
SNAP i.d. Double Well Blot Holder	30	WBAVDBH02
SNAP i.d. Single Well Blot Holder	30	WBAVDBH01

Other Western Blotting Related Products

Forceps	1	XX6200006
bløk-CH Buffer	500 mL	WBAVDCH01
bløk-FL Buffer	500 mL	WBAVDFL01
bløk-PO Buffer	500 mL	WBAVDP001
ReBlot™ Western Blot Recycling Kit	1	2060
ReBlot Plus Kit	1	2500

Immobilon-P PVDF Membrane (0.45 µm pore size) for General Western Blotting Applications

Description	Dimensions (cm)	Qty	Cat. No.
Roll	26.5 × 375	1	IPVH00010
Cut Sheet	26 × 26	10	IPVH304F0
	20 × 20	10	IPVH20200
	15 × 15	10	IPVH15150
	10 × 10	10	IPVH10100
	9 × 12	10	IPVH09120
	8.5 × 13.5	10	IPVH08130
	8 × 10	10	IPVH08100
	7 × 8.4	50	IPVH07850

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call 1-800-MILLIPORE (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

Material Safety Data Sheets (MSDS) are available on our web site. Go to www.millipore.com and enter your catalogue number in the search box.

Standard Warranty

The applicable Millipore Warranty and limited liability for products listed in this publication may be found at www.millipore.com (search on “Terms and Conditions of Sale”).

00112898 Rev. B, 07/10