



Luminata™ Classico Western HRP Substrate

Introduction

Luminata Classico Western HRP Substrate is a premixed, ready-to-use reagent for chemiluminescent detection in western blotting applications that employ horseradish peroxidase (HRP)-conjugated antibodies. Luminata Classico Substrate is ideal for the detection of high to medium abundance proteins. It can be used with traditional immunodetection techniques or the SNAP i.d.® Protein Detection System. Visualization of target proteins is achieved through x-ray film or another imaging method.

Package Contents

Luminata Classico
Western HRP Substrate, 100 mL
Catalogue number WBLUC0100

*covers 1,000 cm² of
membrane area*

Luminata Classico
Western HRP Substrate, 500 mL
Catalogue number WBLUC0500

*covers 5,000 cm² of
membrane area*

Storage/Shelf Life

Store at 2–8 °C. Protection from light is not required. Refer to bottle label for expiration date.

Usage Guidelines

Luminata Classico Substrate is slightly more sensitive than other chemiluminescent HRP substrates, but it is less sensitive than Luminata Crescendo, Luminata Forte, and the original Immobilon® Western HRP Substrates. Compared with these three detection reagents, Luminata Classico Substrate requires a higher concentration of antigen and/or antibodies. See the table below for general guidelines.

HRP Substrate	Luminata Classico	Luminata Crescendo	Luminata Forte	Immobilon Western
Sensitivity	●	●●	●●●	●●●
Antibody Concentration	●●●	●●	●	●

Prior to using this product, review the Material Safety Data Sheet (MSDS) to ensure awareness of associated hazards and use of appropriate controls.

Other Important Considerations

- If switching to Luminata Classico Substrate from a colorimetric substrate, the primary and secondary antibody concentrations may need to be decreased to achieve the optimal signal-to-noise ratio.
- If switching to Luminata Classico Substrate from Luminata Crescendo, Luminata Forte or Immobilon HRP Substrates, the primary and secondary antibody concentrations may need to be increased to achieve the optimal signal-to-noise ratio, or the x-ray film exposure time may need to be increased.
- Optimum exposure time to the x-ray film should be determined for each antibody system.
- Use of blocking buffer to dilute antibodies may reduce background and increase sensitivity.

- To avoid membrane tearing or high background in the blots, always wear gloves and use blunt tip forceps (such as Millipore cat. no. XX6200006) when handling the membrane.
- Never use objects (scissors or forceps) that show visible signs of rusting, since they may create undesirable artifacts or high background areas.
- Do not use sodium azide in any blocking buffers or wash solutions, since it inhibits HRP activity.

Chemiluminescent Detection

Approximately 0.1 mL of HRP substrate is required per cm² of membrane area. The volumes of HRP substrate needed for some common membrane sizes are indicated below:

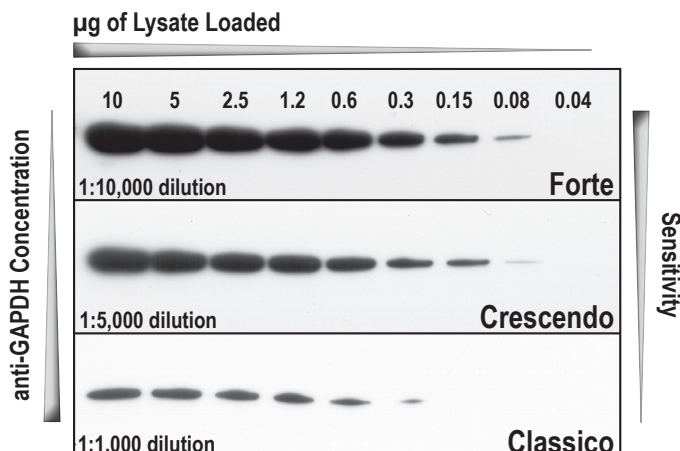
Blot Size (cm)	HRP Substrate Required
7 × 8.4	6 mL
10 × 10	10 mL
8.5 × 13.5	12 mL

1. Place the blot protein-side up in a container or clear plastic sheet protector, and add the HRP substrate onto the blot.
2. Incubate the blot for 2 to 5 minutes at room temperature.
3. Drain the excess substrate, transfer to a clean sheet protector, and cover the blot with plastic wrap or another sheet protector.
4. Expose the blot to a suitable x-ray film for an appropriate amount of time. Typical exposure time for Luminata Classico Substrate is 1 to 5 minutes. The chemiluminescent signal on the blot will last for at least 30 minutes. If higher sensitivity is desired, the detection procedure can be repeated on the same blot, using Luminata Crescendo, Luminata Forte, or Immobilon HRP Substrate.

Proof of Performance

Comparison of Signals from Luminata Forte, Crescendo, and Classico HRP Substrates

Blots of A431 cell lysates (a series of two-fold dilutions ranging from 10 µg to 0.04 µg) were prepared using Immobilon-P membrane and probed with varying concentrations of anti-GAPDH antibody on the SNAP i.d. Protein Detection System. Following incubation with a goat anti-mouse HRP-conjugated secondary antibody, the blots were visualized with Luminata Classico, Crescendo, or Forte Substrate.



Troubleshooting

Symptom	Possible Cause	Solution
High background Negative staining (white bands on black background) Nonspecific bands	Ab concentration is too high.	Expose to x-ray for shorter period of time. Decrease Ab concentration. Use a lower sensitivity HRP detection substrate. Decrease antigen concentration.
Signal disappears quickly in a blot that initially had a very high signal	High HRP-Ab concentration has exhausted the substrate prematurely.	Decrease Ab concentration significantly. Use a lower sensitivity HRP detection substrate. Decrease antigen concentration.
Weak or no signal	Ab concentration is too low.	Expose to x-ray for longer period of time. Use a higher sensitivity HRP detection substrate. Increase Ab concentration. Increase antigen concentration. Change the blocking solution.

Ordering Information

This section lists the catalogue numbers for Luminata Classico 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 Western Blotting and ELISA Applications

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

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").