

# Luminata™ Western Chemiluminescent HRP Substrates

**Chemiluminescent HRP\* substrates** (also known as ECL reagents) are the most sensitive reagents used in the detection of Western blots.

The Luminata Western HRP Substrates are a family of three premixed HRP substrates, which **offer several advantages over other detection reagents:**

- A broad range of sensitivities
- Premixed for more reproducible signals
- Most sensitive substrates in their class

\*horseradish peroxidase



Luminata Classico

Luminata Crescendo

Luminata Forte



Unique Feature	Premixed	Premixed	Premixed
Best used for	Blots where the primary antibody is incubated for ~1 hr	Blot where the primary antibody is incubated > 2 hrs	Blots with overnight primary antibody incubation, or detection of PTM** proteins
Detection Range	~6 pg	~1–3 pg	~400 fg
Signal Duration	1 hr	3 hr	3 hr
Stock Solution Stability	1 yr at 4 °C	1 yr at 4 °C	1 yr at room temperature

\*\*PTM = Post-translationally modified

## Classification of Chemiluminescent HRP Substrates

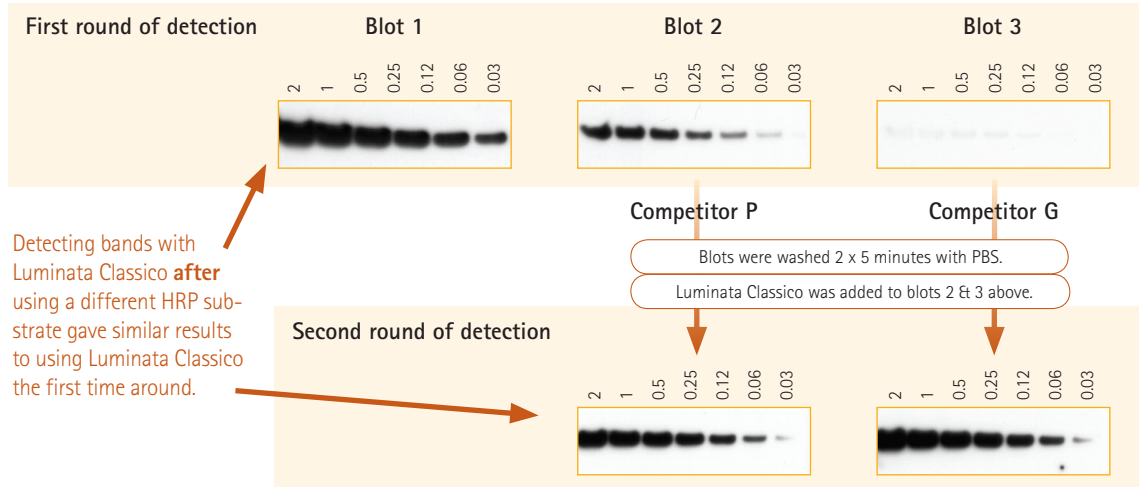
Approximate Detection Limit***	~ 10 pg	~ 1 pg	~ 0.5 – 0.25 pg	~ 0.1 pg
EMD Millipore	Luminata Classico	Luminata Crescendo	Luminata Forte	Visualizer™ Western Blot Kit
Pierce	Pierce ECL	SuperSignal® Pico	SuperSignal Dura	SuperSignal Femto
GE Healthcare	ECL	ECL Plus		ECL Advance
Bio-Rad	Immun-Star™			
Invitrogen	Novex®			
PerkinElmer	Western Lightning® ECL	Western Lightning ECL Plus		

\*\*\*Detection limits obtained from suppliers' published specifications.

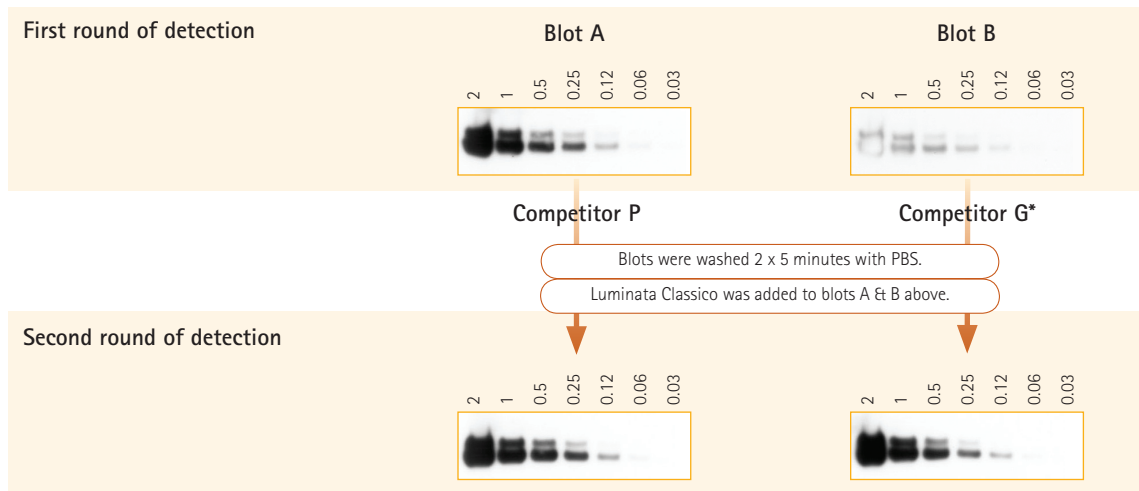
## Test Luminata substrates AFTER your regular HRP substrate

We've tested the Luminata substrates after using other commercial HRP substrates on the same blot and found no significant differences in band intensity compared to detecting with Luminata substrates first.

Try it and you may detect bands you were not able to visualize previously.



**Figure 1. Re-detection of GAPDH.** Three Western blots containing a 2-fold dilution series of A431 extract (ranging from 2–0.03 µg) were probed with 1:1000 dilution of anti-GAPDH (MAB374, EMD Millipore) and 1:1000 dilution of anti-mouse HRP-conjugated secondary antibody (AP124P, EMD Millipore). They were first visualized with the indicated HRP substrate, then washed and re-visualized with Luminata Classico substrate. Blots were exposed to X-ray film for 1 minute.



**Figure 2. Re-detection of ERK1/2 with Luminata Classico.** Two Western blots containing a 2-fold dilution series of A431 extract (ranging from 2–0.03 µg) were probed with 1:200 dilution of anti-ERK1/2 antibody (06-182, EMD Millipore) and a 1:1000 dilution of anti-rabbit HRP-conjugated secondary antibody (AP187P, EMD Millipore). They were first visualized with the indicated HRP substrate, then washed and re-visualized with Luminata Classico substrate. Blots were exposed to X-ray film for 1 minute.

\*Blot B was exposed for 5 minutes for Competitor G's product.

## Obtain the best Western blots possible using Luminata Western HRP Substrates

When no bands were detected with Luminata Classico Western HRP substrate (boxed blot, Figure 3), two choices were available:

1. Test a more sensitive reagent, such as Luminata Crescendo or Forte substrate
2. Increase antibody concentration from 1:10,000 up to 1:1,000



**Figure 3.** Immunoblots containing the indicated amounts of A431 lysate were probed with different concentrations of anti-GAPDH antibody (MAB374, EMD Millipore) indicated in the top row, followed by an appropriate secondary antibody. Bands were visualized using the indicated Luminata HRP substrate and exposed to x-ray film for 5 minutes.

Using higher sensitivity HRP substrates produced the best results and was advantageous in three respects:

1. **Better results:** It produced stronger bands for a more quantitative blot (compare the increase in band intensities for Luminata Crescendo & Forte substrates at 1:10,000 dilution)
2. **Faster:** It took only 10 minutes to wash blot and add a new substrate relative to the 2.5 hours required to repeat antibody incubations.
3. **Cheaper:** The HRP substrates are much cheaper than the price of antibodies.

## Luminata Western HRP Substrates are the most sensitive in their class



**Figure 4.** Comparison of different HRP substrates used during Western blotting. Western blots containing a 2-fold dilution series of A431 extract (ranging from 2–0.03 µg) were probed with either anti-PP2, anti-STAT1, or anti-BRCA1 antibody followed by an appropriate secondary antibody. Blots were first visualized using the class-appropriate HRP substrate from the indicated supplier.

# Ordering Information

## Luminata Western HRP Substrates

Product Description	Quantity	Catalogue No.
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
Visualizer Western Blot Detection Kit, Mouse	250 cm <sup>2</sup> membrane	64-201SP
	1000 cm <sup>2</sup> membrane	64-201
Visualizer Western Blot Detection Kit, Rabbit	250 cm <sup>2</sup> membrane	64-202SP
	1000 cm <sup>2</sup> membrane	64-202

## Immobilon® Transfer Membranes

Product Description	Sizes	Quantity	Catalogue No.
Immobilon P: PVDF, 0.45 µm	7 x 8.4 cm	50/pk	IPVH07850
	26.5 cm x 3.75 m	1 roll	IPVH00010
Immobilon FL: PVDF, 0.45 µm	7 x 8.4 cm	10/pk	IPFL07810
	26.5 cm x 3.75 m	1 roll	IPFL00010
Immobilon PSQ: PVDF, 0.45 µm	7 x 8.4 cm	50/pk	ISEQ07850
	26.5 cm x 3.75 m	1 roll	ISEQ00010

## SNAP i.d.® Protein Detection System

Product Description	Quantity	Catalogue No.
SNAP i.d. Protein Detection System		WBAVDBASE
SNAP i.d. Consumables and Accessories		
Single Well Blot Holder	30/pk	WBAVDBH01
Double Well Blot Holder	30/pk	WBAVDBH02
Triple Well Blot Holder	20/pk	WBAVDBH03
Antibody Collection Tray	20/pk	WBAVDABTR
SNAP i.d. Blot Roller		WBAVDROLL

## BløK™ Noise Cancelling Reagents

Product Description	Detection Reagent Compatibility	Quantity	Catalogue No.
BløK-CH Reagent	Chemiluminescent detection	500 mL	WBAVDCH01
BløK-FL Reagent	Fluorescent detection	500 mL	WBAVDFL01
BløK-PO Reagent	Phosphorylated protein detection	500 mL	WBAVDPO01

## Western Blot Accessory Reagents

Product Description	Quantity	Catalogue No.
ReBlot™ Plus Mild Antibody Stripping Solution, 10x	50 mL	2502
ReBlot Plus Strong Antibody Stripping Solution, 10x	50 mL	2504
Chemilucient™ Plus Western Blot Enhancing Kit	1 kit	2650

## Contact us

In the U.S. and Canada, call toll-free 1 800-Millipore (1-800-645-5476)

In Europe, please call Customer Service:

France: 0825 045 645

Germany: 01805 045 645

Italy: 848 845 645

Spain: 901 516 645

UK: 0870 900 46 45

For other countries across Europe, please call: +44 (0) 115 943 0840,

or visit: [www.millipore.com/offices](http://www.millipore.com/offices).

For Technical Service, please visit [www.millipore.com/techservice](http://www.millipore.com/techservice).



[www.millipore.com/luminata](http://www.millipore.com/luminata)

[www.millipore.com/offices](http://www.millipore.com/offices)