Luminata™ ELISA HRP Substrates

Sensitivity and dynamic detection range for even your most demanding assays

The Luminata ELISA HRP[1] chemiluminescent substrates provide three major advantages:

1. High sensitivity for detecting as little as 0.1 pg of protein.
2. Broad dynamic detection range (up to 2,500-fold), enabling a wide range of quantitation.
3. Flexibility to adjust signal strength by one of two methods:
   a. Exposing the well to the luminometer for a longer time.
   b. Applying a more sensitive substrate to obtain signals in wells with otherwise undetectable signals.



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Key Features of Luminata ELISA substrates

	Luminata Crescendo ELISA	Luminata Forte ELISA	Tetramethylbenzidine (TMB)
Limit of detection[2]	~0.4 pg	~0.1 pg	>2.4 pg
Dynamic detection range	~625x	~2500x	~260x
Signal half-life[3]	>1 hr	>1 hr	Permanent
Stability of working solution (after mixing)	>8 hrs	>8 hrs	N/A
Can re-apply a different substrate	Yes	Yes	No
Shelf life	1 year	1 year	6 months
Incubation time	1–5 min	1–5 min	>15 min

[1]HRP = horseradish peroxidase

[2]Based on IL-2 sandwich assay using 10 µg/ml capture antibody and 0.5 µg/ml detection antibody.

[3]Based on IL-2 sandwich assay using ~16 pg of protein/well.

N/A = Not Applicable

Luminata ELISA HRP Substrates are more sensitive than TMB

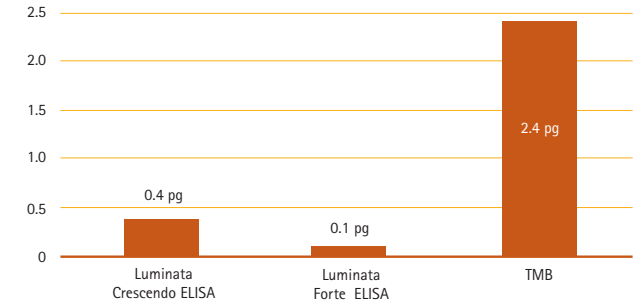


Figure 1. Luminata Crescendo and Forte ELISA HRP substrates are more sensitive than TMB. Detection limits for each substrate were measured with sandwich IL-2 ELISA titration assays using an IL-2 ELISPOT antibody pair (Merck Millipore Cat. No. ELI-002-M). Luminescence was measured using a Wallac VICTOR²™ 1420 counter and TMB color was measured at absorbance 450 nm.

Luminata ELISA HRP Substrates provide up to 2,500-fold dynamic detection range

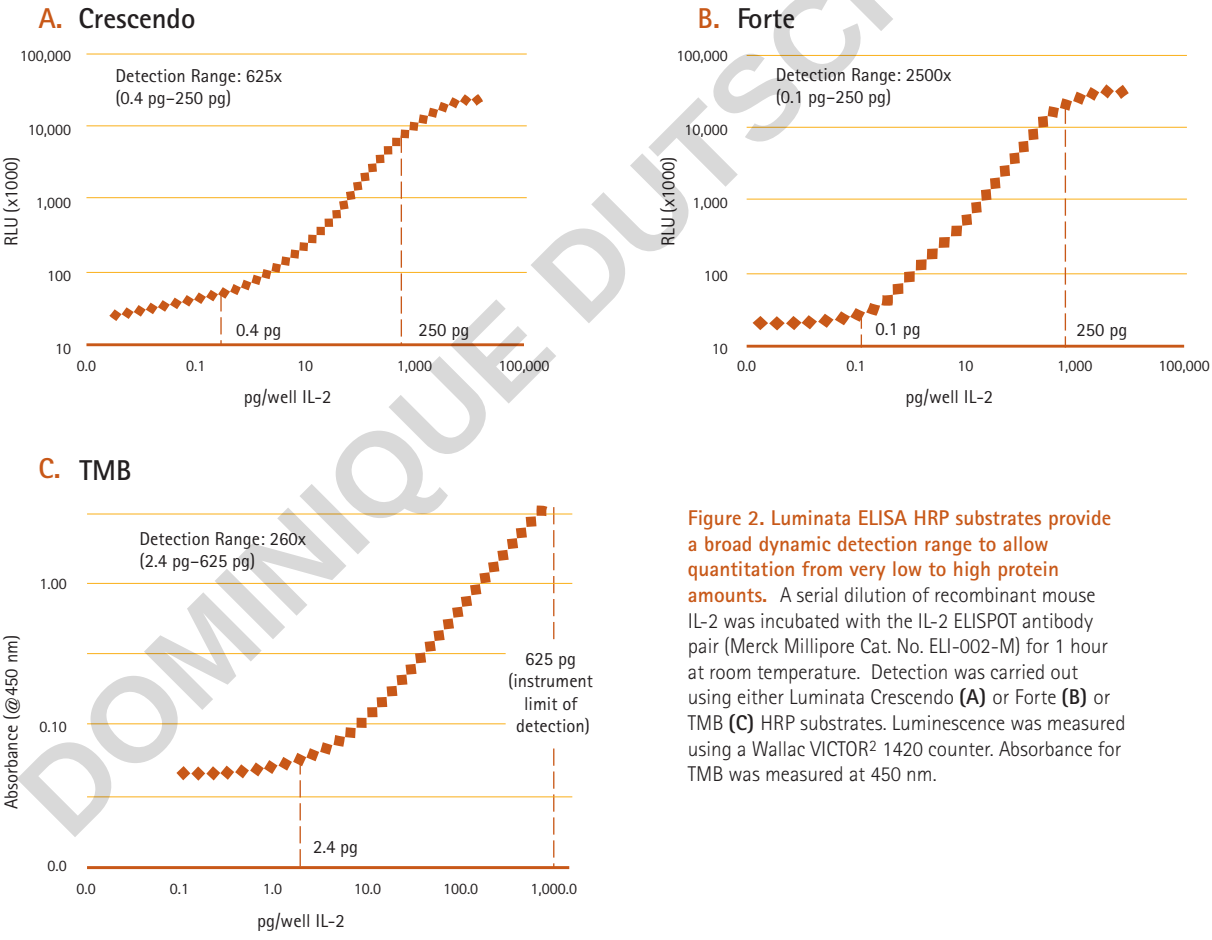


Figure 2. Luminata ELISA HRP substrates provide a broad dynamic detection range to allow quantitation from very low to high protein amounts. A serial dilution of recombinant mouse IL-2 was incubated with the IL-2 ELISPOT antibody pair (Merck Millipore Cat. No. ELI-002-M) for 1 hour at room temperature. Detection was carried out using either Luminata Crescendo **(A)** or Forte **(B)** or TMB **(C)** HRP substrates. Luminescence was measured using a Wallac VICTOR² 1420 counter. Absorbance for TMB was measured at 450 nm.

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Luminata ELISA HRP Substrates are the most sensitive in their class

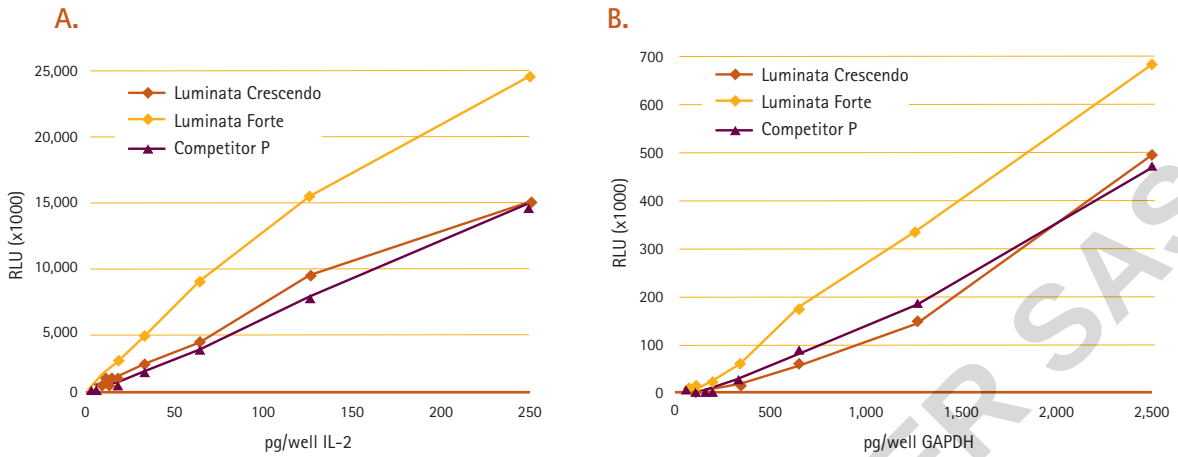


Figure 3. Luminata ELISA HRP substrates offer higher sensitivity than other chemiluminescent ELISA HRP substrates. Three HRP chemiluminescent substrates for ELISA were tested in sandwich IL-2 **(A)** or indirect GAPDH **(B)** ELISA assays. Purified GAPDH (Sigma-Aldrich G5262-1VL) was assayed using mouse anti-GAPDH (Merck Millipore Cat. No. MAB374) and goat anti-mouse IgG, HRP (Merck Millipore Cat. No. AP124P). Luminescence was measured using a Wallac VICTOR^2 1420 counter.

Luminata ELISA HRP Substrates have signal half-lives of > 1 hour

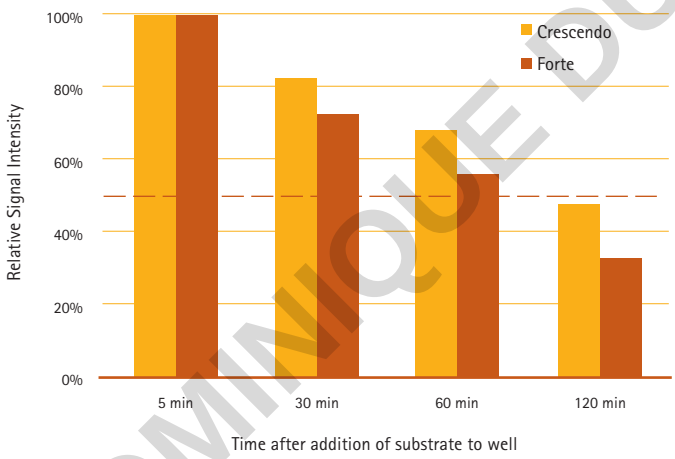


Figure 4. The Luminata ELISA HRP substrates retain > 50% signal one hour after addition to the HRP-containing well. Luminata ELISA HRP substrates were added to an IL-2 sandwich ELISA containing ~ 16 pg of IL-2 and the IL-2 ELISPOT antibody pair (Merck Millipore Cat. No. ELI-002-M). Data were normalized to the signal at 5 minutes. At 5 minutes, Luminata Crescendo ELISA and Luminata Forte ELISA displayed 9.7x10^5 and 30x10^5 RLUs, respectively.

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Ordering Information

Luminata ELISA HRP Substrates

For rapid, sensitive detection using HRP-based ELISA assays

Product Description	Qty	Catalogue No.
Luminata Crescendo ELISA HRP Substrate	100 mL	ELLUR0100
	200 mL	ELLUR0200
Luminata Forte ELISA HRP Substrate	100 mL	ELLUF0100

Chromogenic ELISA AP* Substrates

For permanent visualization using AP-based ELISA assays

Product Description	Qty	Color of Product/ Quantitation Wavelength	Catalogue No.
BCIP/NBT Single Reagent	500 mL	Black, N/A	ES006-500ML
BCIP/TNBT Single Reagent	500 mL	Black, N/A	ES007-500ML
P-NPP Single Reagent	500 mL	Yellow, 405 nm	ES009-500ML

*AP = alkaline phosphatase

Chromogenic ELISA AP Buffers

Pre-made buffers for convenience and optimal results

Product Description	Qty	Catalogue No.
EPA Peroxidase Protection Buffer	500 mL	EPA100
EPA II Peroxidase Protection Buffer	500 mL	EPA102
10X AEC Dilution Buffer For 50X Concentrate	50 mL	ES013-50ML

Chromogenic ELISA HRP Substrates

For permanent visualization using HRP-based ELISA assays

Product Description	Qty	Color of Product/ Quantitation Wavelength	Catalogue No.
ABTS Substrate	100 mL	Green, 410 nm/650 nm	ES004-100ML
ABTS Substrate	500 mL	Green, 410 nm/650 nm	ES004-500ML
DAB Dilution Buffer 10X Concentrate	50 mL	N/A	ES010-50ML
DAB Substrate	10 mL	Black, N/A	ES005-10ML
DAB Substrate	100 mL	Black, N/A	ES005-100ML
TMB/E Substrate	100 mL	Yellow, 450 nm	ES001-100ML
TMB/E Substrate	500 mL	Yellow, 450 nm	ES001-500ML
TMB/E Substrate	1 L	Yellow, 450 nm	ES001-1L-K
TMB/E Ultra Sensitive Substrate	100 mL	Yellow, 450 nm	ES022-100ML
TMB/E Ultra Sensitive Substrate	500 mL	Yellow, 450 nm	ES022-500ML

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