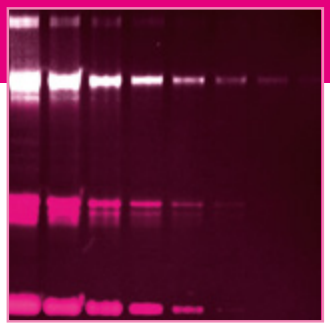
AppliCations
No.9

CheLuminate: Improved Chemiluminescence



Enhanced ChemiLuminescence Detection Kits for Horseradish Peroxidase (HRP) in Western / Southern / Northern Blotting, ELISA and High-throughput Analysis

The peroxidase-catalyzed oxidation of luminol produces a weak flash of light at 425 nm. Incorporation of an electron transfer mediating enhancer forces the flash signal into a glow – and greatly improves the analytical characteristics of the reaction in terms of increased signal intensity and duration [1, 2]. Based on a synergistic effect of primary enhancer and a suitable catalyst, AppliChem's CheLuminate-HRP Kits provide improved chemiluminescence for detection of HRP conjugates in all kind of immunoassays.

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Keywords

Luminol-based

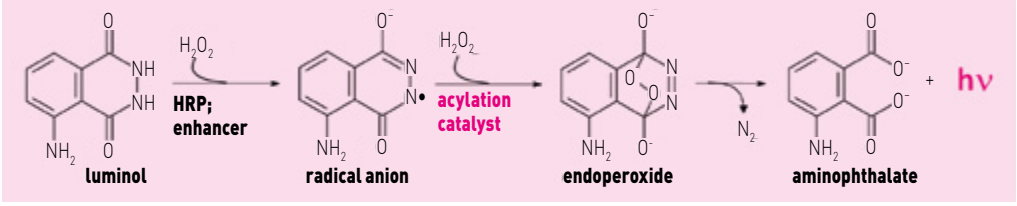
Horseradish Peroxidase

Long light emission

Western Blotting

ELISA

In the presence of hydrogen peroxide (H_2O_2), Horseradish Peroxidase (HRP, EC 1.1.11.7) catalyzes the oxidation of cyclic diacylhydrazides (such as luminol) and acridine derivatives. Immediately following the oxidation, the luminol is in an excited state (intermediate reaction product), which quickly decays to the ground state by emitting light. In contrast, the enhanced HRP-catalyzed oxidation of luminol is a complex multi-step reaction. Enhancers are useful in improving enzyme turnover and increasing the equilibrium concentration of a key intermediate, the luminol radical anion. Conventional systems employ phenolic enhancers only, such as most ECL™ (enhanced chemiluminescence) kits. However, recent work [3, 4] has shown that the light output is significantly increased by addition of a secondary enhancer that functions as an acylation catalyst:



As a result of this breakthrough, the family of AppliChem's **CheLuminate-HRP** chemiluminescent substrates has been developed, with specific formulations to meet the different requirements for **immunoblotting and ELISA**. The **CheLuminate-HRP** kits are ready-to-use two-component systems for chemiluminescent detection of immobilized proteins (Western Blot; ELISA) and immobilized nucleic acids (Southern and Northern Blot) conjugated with horseradish peroxidase (HRP) directly or indirectly.

References

[1] Kricka, L.J. (2000) *Methods Enzymol.* **305**, 370–390.

[2] Heindl, D. & Josel, H.P. (1997) *Non-radioactive Analysis of Biomolecules*, page 258–261. Springer, Berlin.

[3] Marzocchi, E. *et al.* (2008) *Anal. Biochem.* **377**, 189-194.

[4] Vdovenko, M.M *et al.* (2010) *Biotechnology Journal* **5** (8), 886-890.

CheLuminate-HRP for Immunoblotting

Western Blotting is either used to **confirm the presence** of the target protein (by molecular weight and immunological identification) or to additionally **quantify** this information by evaluating the expression level. If Western Blotting is used for absolute quantification (in combination with a calibration curve based on known concentrations of the recombinant protein) or quantification relative to a control sample, **digital image detection is preferred over autoradiography films**. Imaging systems offer the advantages of direct image acquisition and manipulation, higher sensitivity, greater resolution and a wide dynamic range of 3–4 orders of magnitude. Compared to the limits of a film (with a linear dynamic range of 1.5 orders of magnitude), these features allow generation of a high-quality image. Imagers are suitable to quantify both, strong and weak signals, on the same blot – with reliable results. In contrast, on a film a strong signal easily gets saturated, especially with highly intense substrates, bearing the risk of wrong interpretations.

Selection Guide: CheLuminate-HRP Substrates for HRP detection in Immunoblots

	Highly expressed proteins		Medium expressed proteins	Poorly expressed proteins
	Classical ECL™ technology	"New Generation" Kits, employing an additional secondary enhancer		
Product	**A3417 CheLuminate-HRP PicoDetect**	**A7786 CheLuminate-HRP PicoDetect Extended**	**A7807 CheLuminate-HRP FemtoDetect**	**A7879 CheLuminate-HRP FemtoDetect Plus**
Detection Limit	Picogram	Low-picogram (1^{-12})	High-femtogram (10^{-13})	Low-femtogram (10^{-15})
Highlights	• Classic (light output based on phenolic enhancer) • Easy-to-handle • Economical	• Long and steady light emission • Economical	• Extended signal duration (multiple exposure for high quality blots)	• Most sensitive (at least 150 times more intense than standard substrates) • Extended signal duration
Signal Duration	1-2 hours	6 hours	12 hours	8 hours
Suggested antibody dilution*	Primary: 1:100 – 1:5,000 Secondary: 1:5,000 – 1:100,000	Primary: 1:500 – 1:5,000 Secondary: 1:20,000 – 1:100,000	Primary: 1:1,000 – 1:15,000 Secondary: 1:25,000 – 1:150,000	Primary: 1:5,000 – 1:100,000 Secondary: 1:100,000 – 1:500,000
Excellent alternative to	ECL™ SuperSignal®WestPico WesternLightning®PLUS Luminata™Classic Lumi-Light® LumiGLO® LiteAblot®PLUS SERVALightPolaris PicoMax™; PicoTect™ Immun-Star™HRP	ECL™ SuperSignal®WestPico WesternLightning®PLUS Luminata™Classico Lumi-Light® LumiGLO® LiteAblot®PLUS SERVALightPolaris PicoMax™; PicoTect™ Immun-Star™HRP	ECL™Prime SuperSignal®WestDura WesternLightning®PRO Luminata™Crescendo Lumi-Light®PLUS LiteAblot®EXTEND SERVALightEos PCD™ECL WesternBright™ Immun-Star™WesternC™	ECL™Select SuperSignal®WestFemto WesternLightning®Ultra Luminata™Forte LumiGLO Reserve™ LiteAblot®TURBO SERVALightHelios FemtoMax™

*(based on 1 mg/ml stock solution)

The Classic Chemiluminescence Kit: CheLuminate-HRP PicoDetect (A3417)

The CheLuminate-HRP PicoDetect kit corresponds to the classic chemiluminescence substrates based on phenolic enhancers and, therefore, differs from the other CheLuminate products. CheLuminate-HRP PicoDetect is comparable to the ECL™ System in terms of the chemistry and is the substrate of choice for detection of highly expressed proteins in combination with economical (standard) antibodies. Compared to the other CheLuminate-HRP detection kits, CheLuminate-HRP PicoDetect shows the lowest intensity of light output (and therefore bears a low risk of signal-saturation) which simplifies signal detection via autoradiography film. 1–2 hours of light emission allow sufficient time to optimize exposure conditions.

- **Most economic** – Much less costly than other chemiluminescent substrates
- **Superior for film** – For standard Western Blot detection on autoradiography film
- **Easy to handle** – Picogram limit of detection allows detection of highly expressed proteins without background problems
- **High stability** – Working solution is stable for 7 days at RT

A New Generation

The following CheLuminate-HRP detection kits differ from the classical, phenolic enhancer-based CheLuminate-HRP PicoDetect kit. The "new generation" kits are characterized by an acylation catalyst that increases and optimizes the production of light by luminol based systems. **The new patented technology allows controlling the crucial factors of chemiluminescent HRP detection, signal intensity and duration!** The features are:

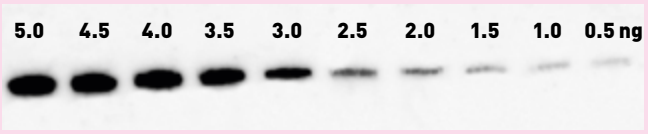
- **Outstanding sensitivity** – Intense light output translates into a corresponding improvement in sensitivity.
- **Long signal duration** – All substrates exhibit long light emission, maximized in the CheLuminate-HRP FemtoDetect substrate.
- **Versatile signal detection** – Detected by film or digital cameras based on CCD (charge-coupled device) sensors. The latter technology offers the advantages of direct image acquisition and manipulation, higher sensitivity, greater resolution and a larger dynamic range.
- **Safe** – None of the components used in CheLuminate-HRP formulations has been reported to be hazardous to human health.
- **Fast** – Substrate is readily prepared by mixing the two components.
- **Stable** – The shelf life of all CheLuminate-HRP stock reagents exceeds 1 year at 2–8 °C. The working solution, obtained by mixing the two components of the kits in a one-to-one ratio, is stable for at least 8 hours.

CheLuminate-HRP PicoDetect Extended (A7786)

CheLuminate-HRP PicoDetect Extended chemiluminescent substrate represents a major improvement over entry-level substrates based on phenolic enhancers. In particular, it offers:

- **Intense light output** – At least 8 times more intense than with phenolic enhancer substrates, such as ECL™ System
- **High sensitivity** – Low-picogram (10^{-12}) limit of detection
- **Long and steady light emission** – 6 hours signal duration allows sufficient time to optimize exposure conditions
- **High stability** – Working solution is stable for 24 hours at RT

The chemiluminescent signal produced by the CheLuminate-HRP PicoDetect Extended substrate can be detected either by film or by imaging equipment based on CCD technology.

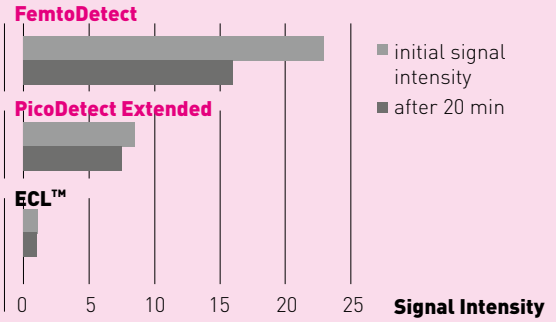


Signal intensity of human transferrin (5 to 0.5 ng) after polyacrylamide electrophoresis, transfer to a PVDF membran and visualization using CheLuminate-HRP PicoDetect Extended. The blocked membrane was incubated with 1:2,000 diluted rabbit anti-transferrin and, after washing, incubated with 1:30,000 diluted HRP-conjugated goat anti-rabbit antibodies. The membrane was washed again and incubated with CheLuminate-HRP PicoDetect Extended. The Blot was acquired using ImageQuant LAS 4000 (GEHC) and 300 s of exposure time.

CheLuminate-HRP FemtoDetect (A7807)

CheLuminate-HRP FemtoDetect substrate is especially formulated to provide very **high light output together with increased light duration**, fully exploiting the advantages of imaging equipment based on CCD cameras. Its key features include:

- **Highly intense light output** – At least 20 times higher than with entry-level phenolic enhancer substrates
- **Amplified sensitivity** – High-femtogram (10^{-13}) limit of detection
- **Long light emission** – 12 hours signal duration allows sufficient time to take multiple exposures for very high quality blots
- **High stability** – Working solution is stable for 24 hours at RT



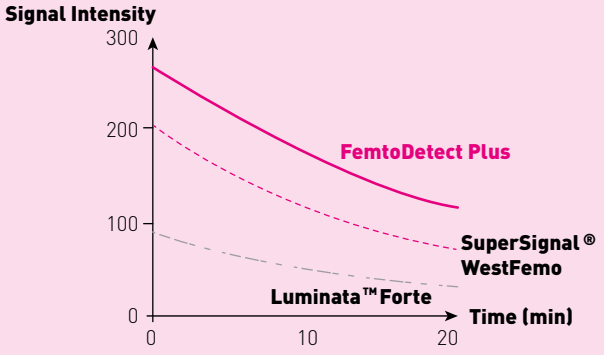
CheLuminate-HRP FemtoDetect combines very high light output with long signal duration. Signal development is performed under standardized reaction conditions and signal intensity is measured in a spectrophotometer. Values are normalized, taking the original ECL™ System (GE Healthcare) as a standard (initial signal = 1).

CheLuminate-HRP FemtoDetect Plus (A7879)

CheLuminate-HRP FemtoDetect Plus is probably the brightest and most sensitive chemiluminescent substrate for HRP available today, making it the first choice chemiluminescent substrate for **detection of very low protein amounts**. Highlights of using CheLuminate-HRP FemtoDetect Plus are:

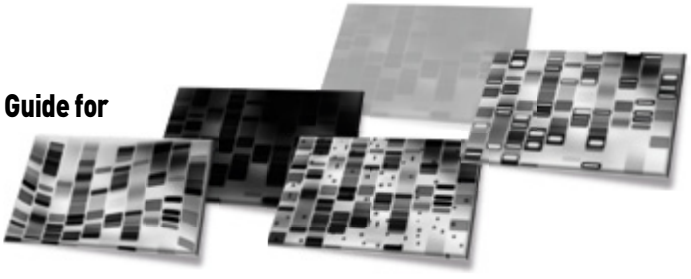
- **Extremely intense light output** – At least 150 times higher than with entry-level phenolic enhancer substrates, or 40–50 times higher than acridan-based substrates
- **Outstanding sensitivity** – Low-femtogram (10^{-15}) limit of detection, allowing the visualization of previously unseen protein bands
- **Long light emission** – 8 hours signal duration allows sufficient time for optimizing exposure conditions

The chemiluminescent signal produced by the CheLuminate-HRP FemtoDetect Plus substrate is preferably detected by imaging equipment based on CCD technology. Film can also be used; however, due to the extremely high light output of this substrate it is important to use very short exposures and/or to further dilute the antibodies.



Relative light output (chemiluminescent signal intensity) of different cheluminescent substrates. Signal development is performed under standardized reaction conditions and signal intensity is measured in a spectrophotometer. Values are normalized, taking the original ECL™ System (GE Healthcare) as a standard (initial signal = 1).

Your blots DON'T HAVE to look like these!
Please check our Optimization and Troubleshooting Guide for Chemiluminescence Detection in Western Blotting:
www.applichem.com/en/products/biochemica/western-blot-elisa



CheLuminate-HRP for ELISA

A bright alternative to colorimetric substrates

In the current analytical practice, ELISA is widely used – most times in combination with HRP. Fields of application are e.g. medicine, pharmacy, food industry, agriculture and microbiology. HRP-coupled immunoreagents allow detection by colorimetric, fluorimetric and chemiluminescent "staining" techniques. In general, chemiluminescence-based ELISAs are the most sensitive, usually doubling the sensitivity of comparable colorimetric assays and giving significantly better results when only small amounts of antigen/antibodies are present.

AppliChem offers two chemiluminescent HRP substrates, based on the new generation CheLuminate technology and **optimized for ELISA**. Our products provide

- **Immediate light generation** – An intense signal is generated immediately at RT or 37°C
- **Outstanding sensitivity**
- **Wide dynamic range** – No need to dilute samples
- **Excellent low-end linearity** of dose-response curves (reading after 1 min)

CheLuminate-HRP Substrates for ELISA and High-Throughput Screenings

Product	A8055 CheLuminate-HRP ELISA FemtoDetect	A8031 CheLuminate-HRP ELISA FemtoDetect Plus
Detection Limit	Mid-femtogram (10^{-14})	Low-femtogram (10^{-15})
Features	• Extended signal duration • Optimized for test tube or microplate luminometer based applications • Excellent low-end linearity of dose-response curves	• Most sensitive (at least 150 times more intense than standard substrates) • Optimized for high-throughput microarray assays • Excellent low-end linearity of dose-response curves
comparable to	WesternLightning®PRO Luminata™Forte ELISA Lumi-Light®PLUS	SuperSignal®ELISAFemto WesternLightning®Ultra Luminata™Forte ELISA LumiGLO Reserve™ QuantiGlo®

CheLuminate-HRP ELISA substrates are perfectly suitable for all kind of ELISA or microplate readers, also with automated liquid handling systems!

CheLuminate-HRP and related products

Prod. No.	Description	Details
A3417	CheLuminate-HRP PicoDetect	Sufficient for 1200 cm² / 5000 cm² / 10000 cm² of membrane
A7786	CheLuminate-HRP PicoDetect Extended	Sufficient for 500 cm² / 2500 cm² / 5000 cm² of membrane
A7807	CheLuminate-HRP FemtoDetect	Sufficient for 500 cm² / 2500 cm² / 5000 cm² of membrane
A7879	CheLuminate-HRP FemtoDetect Plus	Sufficient for 200 cm² / 1000 cm² of membrane
A8055	CheLuminate-HRP ELISA FemtoDetect	Sufficient for 5 x 96-well plates
A8031	CheLuminate-HRP ELISA FemtoDetect Plus	Sufficient for 5 x / 10 x 96-well plates
related products		
A5239	Pure Nitrocellulose unsupported 0.45 µm Transfer Membrane	Nitrocellulose membrane; different sizes available
A5243	PVDF-Star Transfer Membrane 0.45 µm	Polyvinylidenfluorid membrane; different sizes available
A1391	Albumin Fraction V (pH 7.0)	BSA, bovine serum albumin
A7099	Blocking Buffer I	Based on chemically modified, low-molecular weight casein; for blocking of unspecific binding sites
A7516	Blocking Buffer II EGrade	Protein-based; cost-effective alternative to Blocking Buffer I
A6485	CrossDown Buffer	Used as sample buffer or antibody dilution buffer for reduction of unspecific binding, cross-reactivities and matrix effects
A1389	Tween® 20 BioChemica	Non-ionic detergent, common component in immunoassay-buffers, decreases the nonspecific binding of antibodies
A5001	TBS (Tris-buffered saline) (20X) – Powder	Buffer base for blocking, washing and antibody incubation of immunoblots
A9201	PBS tablets pH 7.4 (for 1 L)	Buffer base for blocking, washing and antibody incubation of immunoblots

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