FT-36349A

UptiLight™HS ELISA HRP substrate

Product Description

Chemiluminescent substrate solution for the detection of immobilized peroxidase.

Name :	**UptiLight™ HighSensitivity ELISA HRP chemiluminescent substrate**			
Catalog Number:		**36349A**, 200 ml	**36349B**, 500 ml	**36349C**, 2 L
Product Components:	Uptilight **Reagent A**	100 ml	500ml	1L
	Uptilight **Reagent B**	100 ml	500ml	1L

Storage : +4°C, avoid direct light (L). Stable for a minimum of 18 months when stored at +4°C.

*** For use only in enzyme linked immunosorbent assays (ELISA) - Not for membrane based assays. ***

Introduction

The detection of immobilized peroxidase was popularized by immuno-assays: nitrocellulose, nylon or PVDF sheets (blots) or microplates (ELISA). Overcoming the performance (and first, the sensitivity) of classical insoluble chromogenic substrates (4-CN, AEC, TMB, DAB), the luminol was introduced as a convenient and effective chemiluminescent substrate. The principle is that the by products of the chemical reaction of peroxidase with H_2O_2 and luminol generates light. In ELISA, the emitted glow is then recorded by a luminometer at 425nm in the wells of an ELISA microplate. The use of luminescent substrates is most recommended for quantitative assays requiring an extended dynamic range (wide range of detection) or qualitative assays requiring the best achievable detection limit (highest sensitivity). Especially in screening experiments, one crucial point relies on the reagent stability and batch to batch reproducibility.

Uptima provides HRP chemiluminescent substrate formulations optimized for ELISA applications (and also for Western-Blotting), with 2 sensitivity levels, High (HS) and Ultra (US): UptiLight™HS #36349A is the cost-effective version for the ELISA of antigens up to picogram ranges, recommended for standard applications; UptiLight™US #99620 is designed for detection up to the femtogram range in the most demanding applications. The UptiLight ELISA substrate is very sensitive and reproducible. However great care must be taken to optimize the individual assay components (antibodies, conjugates, solid phase, etc.) to minimize background reactivity associated with non-specific immunochemical reactivities.

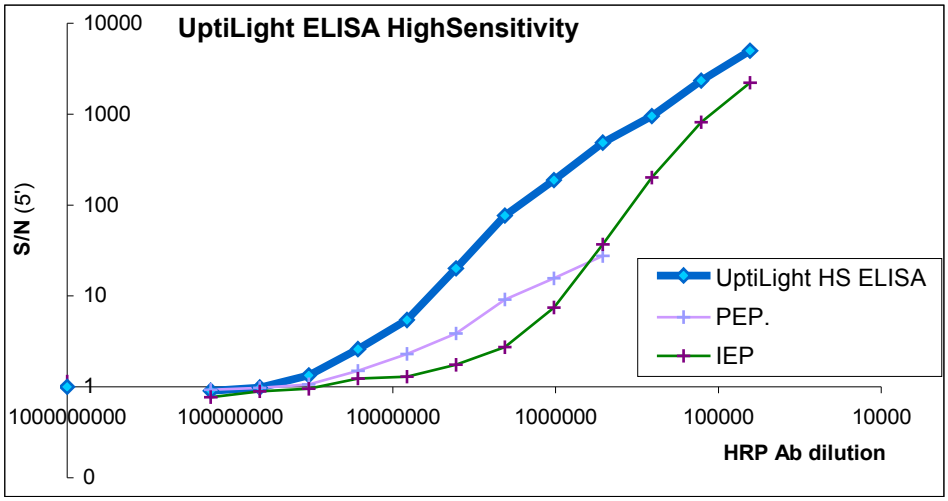

Figure A: Signal to noise in ELISA with UptiLight™HS ELISA HRP substrate (#36349A) compared with competitors PEP and IEP. ELISA was performed with coated Mouse IgG detected by anti Mouse IgG(H+L) - HRP (#UP446330), then the ECL Luminescent substrates, prepared according their respective supplier. Luminescence was recorded with Mithras (Berthold Technologies) with 0.1sec integration time, after a 5min pr-incubation period. Data plotted as Signal to Noise ratios (S/N) for each tested HRP Ab concentration. Reduced background and higher sensitivity was found with UptiLight™HS.

Directions for use

Handling and Storage

The reagents are stable for a minimum of 18months when stored at 2-8°C in the original container and protected from light.
Use only clear recipients: use disposable test tubes for small volumes. If recipients should be used again (beaker), wash them with suitable cleaning agent and rinse well with distilled water. Traces of metals, immunoreagents, or detergents may affect results.
Use white or black microplates, to avoid well-to-well cross-talk.

Guidelines for ELISA with UptiLight detection

UptiLight™HS ELISA HRP substrate is optimized for standard Enzyme-Linked ImmunoAssays in microplates (ELISA). ELISA immunoassays can be performed according to standard procedures. Because of UptiLight substrates high sensitivity, great care must be taken to optimize the individual assay components (antibodies, conjugates, solid phase, etc.) to minimize background reactivity associated with non-specific immunochemical reactivities. Modification of substrate protocol (substrate incubation duration, recording time,...) may eventually be required for specific applications, or for other luminescent assays (please ask Uptima, about our other UptiLight reagents).

Microplates Preparation	Perform the ELISA procedure as usual. Refer below for optimizations. Recommended microplates are FPLyte polystyrene 96 or 384 well microplates.
Coating:	*i.e. protein or primary antibody at 1-10µg/ml in carbonate buffer pH9.6*
Saturation:	*5% BSA (or 5% fatty free milk, Tween20® 0.1%, or SeaBlock #UP40301A)*
Wash:	*3 times with 250µl PBS+Tween20® 0.01%*
	Coated/saturated microplates can usually be kept protected by a Saran® wrap at 4°C for weeks

Probes:	Incubate each reagent successively *-Direct ELISA: Primary antibody then secondary labeled antibody; other probes include (strept)avidin lectins, nucleic acid…* *-Sandwich ELISA: sample to analyze (capture), then second probe (HRP labeled antibody)* *-Inhibition ELISA: sample + HRP labeled tracer*
dilution buffer:	PBS +Tween20® 0.01%, followed by a wash step
incubation:	100µl per well for 1H at 37°C

Washes	4 times with 250µl per well of PBS+Tween20® 0.01%
Final wash	1 time with 250µl per well of PBS; empty well the wells

Uptilight Substrate Prep.	Prepare the working solution by mixing **1 part reagent A** with **1 part reagent B**, in sufficient volume. Typically, prepare 10ml/microplate. Mix well and protect from light. Allow to reach room temperature.
Incubation:	Add 100 µl of UptiLight working substrate per well. Incubate for 5 min.
Reading:	Read using a luminometer (425nm, 0.2-1 sec integration time) or alternative method.

PBS = Phosphate Buffered Saline

Technical information

- UptiLight™HS ELISA HRP Substrate, produced according to strictly controlled procedures, gives highly sensitive and reproducible results. Inconsistent results may be caused by small changes in immunoassay operating, protocol and reagents quality. A crucial point for optimal results relies on keeping the right probe concentration and saturating agents, for the lowest background as possible whilst maintaining good signal.
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- For critical applications, several **optimization approaches** can be carried out to improve the net signal:
  -optimize antibody concentration (test 2-10-fold series dilutions). Take advantage of lower concentrations (that decrease the background while keeping good signal), than with higher concentrations (that increase signal, but may unfortunately also increase background),
  -optimize the saturating agent, or antibody buffers (test higher & lower saturant and buffer concentrations). This is often the most successful approach combined with antibody concentration optimization.
  -optimize the UptiLight pre-incubation time, and the light recording time.

- The **dilution of antigen, primary and secondary probes** (for example antibodies) must often be 5-50fold higher than with conventional chromogenic detection systems (OPD, TMB), resulting in a saving of reagents, while improving sensitivity.
- The background is very low, using immunology grade quality reagents. We recommend using **standard buffers** as outlined above for each step. 3 washes are generally sufficient, but we recommended washing finally with PBS (no Tween20), and to empty the wells, by tapping the plate on absorbing paper. When a standard buffer is not providing satisfactory results, firstly check you have the correct immuno-reagent concentrations, then you may try to increase the saturating concentration, include different saturating agents, or a higher salt washing buffer (0.5M NaCl). 5% BSA for saturation can be replaced by other saturating materials. 1/5th to 1/10th of the saturating agent can be included in reagent dilution buffer (i.e. PBS + Tween®20 0.01% + 0.1% BSA)... but there is no universal system: the best buffer or saturating agent depends on the antigen, antigen/probe affinity, detection system.

  Background may be caused when using :
  -milk based saturating agents, as they may contain endogenous biotin, a natural vitamin, that can generate an unspecific signal with (strept)avidin detection systems.
  -saturants and buffers prepared with metallic (fericyanure, cobalt, copper) or other compounds (hematin),
  -contamination of buffer or reagents may catalyze the chemiluminescent reaction.
- The microplate **luminometer** (medium shaking intensity, integration time 0.1s) should be set up according to manufacturer recommendations. UptiLight is controlled with Mithras instrument (Berthold Technologies).
- The detection **sensitivity** of HRP is excellent, and was found higher than other commercial ECL reagents (see Figure A). Pico to femto grams of antigens can be detected.
- UptiLight®HS emits very **stable luminescence** (see figure B). Reading can be performed with 0.1-10sec integration time, for up 60minutes. Background may decrease more rapidly than signal, as shows figure B for high HRP concentrations, but usually S/N are not improved for lower concentrations that are generally more critical.

  It is recommended to record light output for a short period, typically 0.1sec, as higher times usually do not improve sensitivity even increasing signal.

  5min **pre-incubation** is recommended as it was found optimal to set the maximum signal while minimizing the differences due to distribution time between first to last wells of microplate. In your conditions, lower or higher pre-incubation time could yield more favorable signal / background and more accurate inter-well reproducibility. Background may decrease more rapidly than signal (higher S/N shown on figure B for high HRP concentrations), but usually S/N are not improved for lower HRP concentrations that are generally more critical.

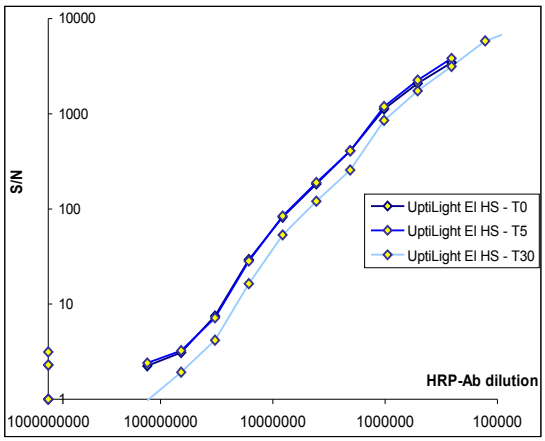

Figure B: Photostability of light emission with UptiLight HS ELISA HRP Substrate. Luminescence was recorded with 0.1sec integration time after a 1, 5 or 30min pre-incubation period.

- UptiLight substrate, with its high sensitivity and stable luminescence, meets the **requirement of HTS applications** especially, where microplate handling could require a long and variable time, and microplate reading should be performed rapidly. The working reagent (A+B) is stable enough (at least 1H) without compromising sensitivity; plates can be read very fast, with optimal reproducibility inter- and intra-experiments.

References

- **Briot A.** *et al*, Kallikrein 5 induces atopic dermatitis–like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome, *J. Exp. Med*., 10.1084/jem.20082242 (2009) - Mithras

Other information

Related products

UptiLight ELISA #996201 (ultra-sensitive))
SeaBlock agent #UP40301A
TBS with non fat milk #GS4160
TBS with Tween20 #UPGS4200
BSA Biotech grade #UPQ84170 (powder) or #UP900130 (solution 30%)

For any questions, please ask Uptima

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