FT-58372A

UptiLight™US HRP WB Chemiluminescent Substrate

Product Description

Luminol based chemiluminescent substrate solution for the detection of immobilized peroxidase.

Name :	**UptiLight UltraSensitive HRP Blot chemiluminescent substrate** (Femto Range detection)			
Catalog Numbers :		58372A, 60ml for 540 cm^2	58372B, 120ml * for 1080 cm^2	58372C, 300ml for 2 700 cm^2
Product Components :	Uptilight **Reagent A**	1x20 ml	1x40 ml	1x100 ml
	Uptilight **Reagent B**	2x20 ml	2x40 ml	2x100 ml

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

* sufficient for 20-30 miniblots

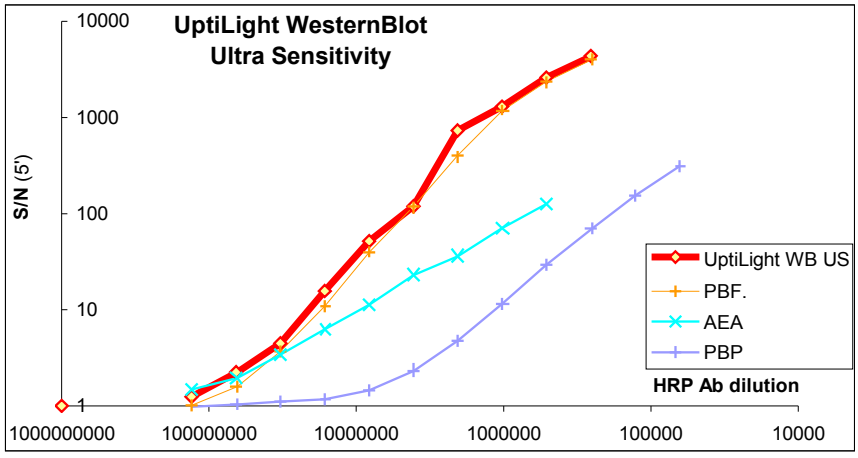
*** For use in enzyme linked membrane based assays. ***

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 . 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). In Blotting applications, the emitted glow is then recorded by exposure to radiographic film, or measured by CCD camera or a scanner.

Uptima provides HRP chemiluminescent substrate formulations optimized for Western-Blotting (and ELISA) applications. UptiLight™ US HRP WB #58372A is designed for detection of antigens in the Femtogram range for the most demanding of applications. (Other formulations are available for lower and intermediate sensitivity, and one-component/spray reagent).

- **Ultimate sensitivity**: detect less than 1 femtogram !
- **High resolution**: highest signal with minimal background
- **Wider dynamic range**
- **Long signal duration**
- Requires **lowest quantities** of antigen and antibodies



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 or immunoreagents may affect results.

Guidelines for WesternBlotting and ImmunoAssay with UptiLight detection

UptiLight HRP WB substrate is optimized for Western Blotting using Nitrocellulose or PVDF blotting membranes. Prepare the blot and immunoassay according to standard procedures. Great care must be taken to optimize the individual assay components (antibodies, conjugates, saturant, etc.) to minimize background associated with non-specific binding. Refer to the standard procedure in the following table and below for additional recommendations.

Preparation of the Blot	Perform the Blotting procedure as usual. See below for further recommendations. Recommended membranes are Nitrocellulose and PVDF. .
Saturation	5% BSA, or 5% fatty free milk, Tween20® 0.1%, or SeaBlock (#UP40301A)
Wash	3 times with 20ml PBS+Tween20® 0.01%
Probes	Incubate all probes successively 1H at +37°C, followed by a wash step *All diluted in PBS +Tween20® 0.01%* *Specific probe (Primary antibody, nucleic acid…)* *Peroxidase labeled secondary agent (or probe)*
Wash (final)	Rinse the blot briefly, then Wash 3 times with PBS+Tween20® 0.01% (5-10min, 20ml per blot each) Finally wash 1 time 10min with PBS; drain off excess reagent
Uptilight Substrate prep.	Prepare the working solution by mixing **1 part reagent A** with **2 parts reagent B**. Sufficient volume required to cover the blot is typically 0.111ml/m^2, i.e. 4ml/miniblot. Mix well and protect from light. Allow to reach room temperature.
Incubation	Add UptiLight working substrate onto the blot, homogeously. Incubate for 1 min without agitation, and drain off excess reagent.
Radiography	Expose the blot to a radiographic film for 1-30min (see below), or read the blot with a CDD camera or a light scanner.

Note: modification of substrate protocol (substrate incubation duration and T°, recording time,...) may be required for your specific application, other blots such as Dot-, Northern- and Southern- blots.. But most generally, optimization should be carried out at steps of blot preparation and detection.

Technical information

- UptiLight UltraSensitivity HRP WB Substrate is produced according to strictly controlled procedures and gives highly sensitive and reproducible results. Inconsistent results may be caused by small changes in operating immunoassay reagents quality.

- A critical point for optimal results relies on keeping the right probe concentrations with the lowest background. The dilutions of antigen, primary and secondary probes (for example antibodies) must often be 10-100 fold higher than those used for conventional chromogenic detection systems (OPD, TMB), resulting in a saving of reagents, while increasing sensitivity.

- The **background** is very low using immunology grade quality reagents. We recommend using standard buffers as outlined above. When a standard buffer is not providing satisfactory results, firstly check you have the correct immuno-reagents concentrations, then you may try to increase a saturating agent concentration, include different saturating agents, or a higher salt washing buffer (0.5M NaCl). I.e. 5% BSA for saturation can be replaced by other saturating materials. 1/10-20th of saturant concentration can be included in reagent dilution buffer (i.e. PBS + Tween®20 0.01% + 0.1% BSA)... but there is no universal optimal buffer system:
  Background may be caused by:
  -Milk and BSA based saturating agents may contain traces of immunoglobulin that will generate a strong background with the anti IgG secondary antibody (even against non-bovine Igs, by crossreaction). To that point, we recommend our BSA Biotech grade #UPQ84170 (powder) or #UP900130 (solution 30%).
  -Milk based saturating agents, may contain endogenous biotin, a natural vitamin, that can generate unspecific signal with (strept)avidin detection systems; We recommend our BioBlock agent # N13650.

-saturants and buffers prepared with metallic (fericyanure, cobalt, copper) or other compounds (hematin, Ig materials), as well as contaminants, may catalyse or interfere with the chemiluminescent reaction. It is for example necessary to use Ig free BSA.

-bacterial contamination may alter antigens or probes or saturating agents

- **Antigen masking** may occur with certain saturating agents. Again, there is no universal optimal saturant: one saturating agent may be excellent or good for antigens A, B and C, but hide a defined antigen X, while a second saturant will be found the best for antigen X, good for A, mediocre for B and bad for C. So the best buffer or saturating agent should ideally be determined depending on the antigen, antigen/probe affinity, detection system.

- The blot should be incubated with the correct **reagent volumes** (antibody, washes, UptiLight) under proper **agitation**. Uni-directional shaker, too slow or too quick agitation, insufficient volumes,... may cause uneven background, and lower signal.

- **X-Ray film exposure.** The following steps should be performed in a dark room (red safe lights). ECL light emission occurs at 425-510nm rapidly, often visible by eye as a glow, reaching its maximum within 5 minutes. 1 minute incubation is sufficient. Drain off excess reagent by holding the membrane vertically and touching the edge of the membrane against an absorbing paper, and place it a radiographic cassette. Wrap membrane blot in transparent plastic film (Saran® wrap) or alternative suitable pocket, in a radiographic cassette. Remove bubbles avoid applying pressure. Place an X-ray film over (it must not get wet!) and close the cassette for 1-30min. Do not move the film whilst it is being exposed. After the desired exposure time, again in dark room remove the X-Ray film to be developed. Start with a short exposure time, i.e. 1min, to appreciate the staining level. The time of a new radiographic exposures can be adjusted, from the previous results, for best results and multiple copies.

Related products

See our our BioScience catalog for:

*other reagents for immunoblotting with UptiLight HRP
HRP-labeled secondary antibodies (p.A324)
HRP labeled (strept)avidins (p.A350)
ProTran 0.2µm NC membranes, 20x20cm, #S31441
Blotting paper 1mm thick, 460x570cm, #BP2791
Stains: Protein Membrane Reversible stain #20078A
Antibody Stripping Buffer, #L7710A

buffers and saturants (A365): SeaBlock agent #UP40301A
TBS buffer UP74004A (also available with non fat milk #GS4160, with Tween20 #GS4200,...)
BSA #UPQ84170 (powder) or #UP900130 (soln 30%)
BioBlock Saturating agent for blotting (inTBS) #N13650
Non fat milk powder #768701

*other chemiluminescent substrates for blotting:
UptiLight classic HRP WB Substrate #UP996190
UptiLightOne HRP WB Substrate, spray #BM4961/BM4963
UptiLight HighSensitivity HRP WB Substrate #98490A
VisGlo AP WB Substrates #BV3021, #BV3031

*other substrates for HRP ELISA :
UptiLight HRP ELISA Chemilum. Subst.#99620&36349A
TMB HRP ELISA Chromogenic Substrate #664780(soln)

Other information

For any questions, please ask Uptima

Registered trademarks: Tween®, from ICI Americas; Saran® from Dow Chemical

rev.:I03E-G07E