FT-99619A

UptiLight™ HRP WB Chemiluminescent substrate

Luminol based chemiluminescent substrate solution for the detection of immobilized peroxidase (Western-Blotting)

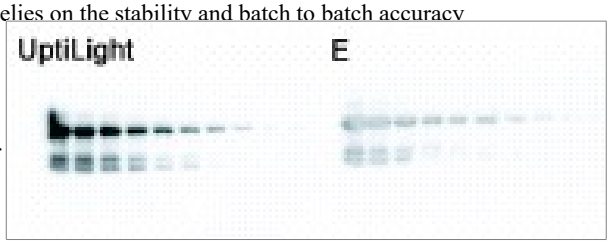
Description

Product Number	**Description**
UP99619A	UptiLight HRP blotting chemiluminescent substrate
	Contains Reagent A (2x250ml, Luminol) Reagent B (2x15ml, oxidizer) [sufficient quantity for the detection of 4500cm^2 - 80 miniblots (7x8cm)]
UP99619D	UptiLight HRP blotting chemiluminescent substrate * Special Packaging *
	Contains Reagent A (20x8ml, Luminol) Reagent B (10ml, oxidizer) [sufficient quantity for the detection of 20 miniblots (7x8cm)]

Storage : +4°C, avoid direct light
Stability: 1 year from purchase date, when stored according to the recommended storage conditions

General Considerations

The detection of immobilized peroxidase was popularized by immuno-assays: nitrocellulose, nylon or PVDF sheets (blots), where samples are immobilized, and probed with several reagents, the last step consisting of enzyme labeled reagent. 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 consists of the generation of light by the by-products of the chemical reaction from peroxidase upon the substrate. The emission of light is then recorded by a radiographic film, or a CDD camera. One crucial point relies on the stability and batch to batch accuracy of the reagent.



Uptima developed a formulation for WB applications, optimized to give good sensitivity of detection for standard analysis, that is ready and easy to use, stable, and economical: UptiLight UP99619 ensures quality images for your HRP blots, with strong bands and low background. It allows for direct scanning recording and for multiple records by autoradiography.

Technical information

- The sensitivity of detection in HRP WBblots is very high: UptiLight was successfully used to detect as low as 1pg of mouse IgG with a peroxidase labeled anti mouse secondary antibody. Sensitivity is improved with radiographic exposure time, thanks to a prolonged emission rate.
- A crucial point for optimal results relies on keeping the right probe concentrations with a low background. For that reason, the dilutions of antigen, primary and secondary probes (for example antibodies) must often be higher than with conventional detection systems, resulting in a saving of reagents, without impairing sensitivity.
- The background is very low under the recommended protocol, using immunology grade quality reagents. It may however be increased when using unsuitable reagents, for different reasons:

- milk based saturating agents may contain endogenous biotin, a natural vitamin, that can generate an unspecific signal with (strept)avidin detection systems.
- Saturants and buffer prepared with metallic (ferric, cobalt, copper) or other compounds (hematin), -contaminated chemicals, may catalyse the luminol reaction.

- UptiLight has been used successfully with the following chemiluminescent detection scanners: Fuji (Ray technology), Science technology (Chemimager), Berthold Technology, Perkin Elmer.
  Note: It works in ELISA, but for this application we recommend our optimized formulation UptiLight ELISA #36349 or #99620.

Directions for Use

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 the results.

The following protocol of blotting is given for a standard miniblot 8x8cm^2. The choice of temperature and duration of incubation, and the antibody and saturating buffers may be modified for special applications. Ask Uptima for blot handling precautions (see troubleshooting).

Protocol:

Preparation of the blot	.Perform the blotting steps according to usual procedures: **Western, Northern and Southern blotting:* separation of molecules (proteins, nucleic acids) by electrophoresis, then transfer onto nitrocellulose sheets **Dot blotting:* antigens deposited on spots Notes: Take care using immunological grade reagents Compatible blotting membranes: nitrocellulose, PVDF, nylon, Magna…
Saturation	.5% fat free milk, Tween20® 0.1%, or SeaBlock (#UP40301A) in PBS (or TBS) Note: combine milk 5% and Tween20 0.1% suits most applications. The use of 5% BSA is possible, but not recommended in the first instance .incubate for 1 H at +37°C (alternate convenient mode: overnight at +4°C)
Wash	3 times for 5 min 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% Probe diluted in PBST (Primary antibody, nucleic acid probe) Peroxidase labeled probe
Wash	Rinse briefly, then wash: 2 times for 5 min with 20ml PBS+Tween20® 0.01% 1 time for 10 min with 20ml PBS+Tween20® 0.01%
final wash:	1 times for 5 min with 20 ml of PBS (prepare the substrate)
Substrate	Allow UptiLight reagent to reach room temperature, avoid direct light. Sufficient volume required to completely cover the blot is typically 0.111ml/m^2, i.e. : Put 8ml of reagent A for a miniblot 7x8cm^2 in a clean 10x10cm box Add 8 drops (ca 400µl) of reagent B, mix
Incubation	Drain the blot from excess PBS Transfer to a bath of UptiLight working solution, homogenize, incubate for 1 min
Radiographic staining	Drain the blot from excess reagent Put it in a radiographic cassette, and cover with a clean plastic film
	In a dark room: switch off the light Cover the blot with a radiographic film and expose 1-30min* Stain the radiographic film in developing then fixating agents *Note: First exposure can be done during 1min to appreciate signal level: the time of further radiographic exposure can be adjusted for best results and multiple copies.
	The blot can be kept for other types of analysis. In case of reprobing with different antibodies, it may be useful to strip beforehand the first antibody with reagent #L7710A.

Other information

Trouble Shooting

Problem	Causes	Answer
Background is too high, and homogenous, image is reversed on film, brown or yellow bands	Antibody concentration is too high	dilute your primary and/or secondary antibody
	Buffer	Prepare fresh buffers, change the saturating agent
	Insufficient washing	Increase the duration of wash, ensure that the buffer is completely removed before adding fresh buffer
	Exposure	Drain off excess reagent before exposure to X-ray film. Reduce exposure time of the blot with the radiographic film
	Unsuitable membrane	Use another type of membrane (NC)
Background is too high, but heterogeneous	Traces	Take care of membrane handling (wear gloves to avoid skin contact)
	Dots and zones	Check the saturating agent is well dissolved; check that there is enough reagents solution to completely cover the blot with constant agitation
No or weak signal	Transfer	Check proteins are correctly transferred to the blot by reversible staining (20078A); Put more protein if needed.
	Reagents	Try to dilute the HRP-conjugate (that is in excess and quench the substrate) Try another antibody (higher affinity) Try our UptiLight WB #98490A (high sensitivity) or #58372A (ultimate sensitivity)

Note: UptiLight Classic is stable for 1 year under normal conditions of use (even if the bottle reaches room temperature).

Related products:

UptiLightOne HRP WB Substrate, spray #BM4961, Dropper #BM4963

UptiLightUS HRP WB Substrate #58372A (femto range detection)

UptiLight HRP ELISA Substrates #36349A (pico range detection) and #996201(Femto range detection)

Protein MW markers for ECL #UP344440 (incl. a blue marker for electrophoresis, and blotting-positive control/SAV-HRP)

Protein Membrane Reversible stain #UP20078A

SeaBlock agent #UP40301A

TBS with non fat milk #GS4160 or TBS with Tween20 #UPGS4200

BSA Biotech grade #UPQ84170 (powder) or #UP900130 (solution 30%)

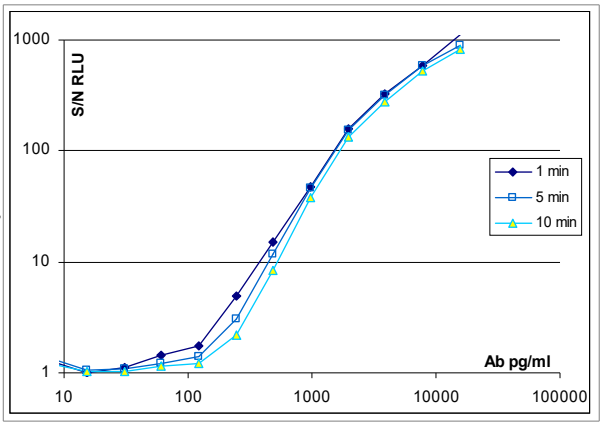
BioBlock Saturating agent for (W, N, S) blotting (in TBS) #N13650

Non fat milk powder #768701

ProTran 0.2µm NC membranes, 20x20cm, #S31441

Blotting paper 1mm thick, 460x570cm, #BP2791

Antibody Stripping Buffer, #L7710A



UptiLight UP99619 test with HRP anti Mouse IgG #UP446330 on coated Mouse IgG.

References

- **Brandt K.** *et al.*, A novel MEK2/PI3Kδ pathway controls the expression of IL-1 receptor antagonist in IFN-β-activated human monocytes, *J. Leukoc. Biol.*, 88: 1191 - 1200 (2010) Article
- **Buey R.** *et al.*, Sequence Determinants of a Microtubule Tip Localization Signal (MtLS), *J. Biol. Chem.*, 287: 28227 - 28242 (2012) Abstract

For any question, please ask Uptima - Interchim

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