UptiLight HRP blot substrate

Chemiluminescent substrate for blotting with peroxidase

Product Description

Luminol based chemiluminescent substrate solution for the detection of immobilized peroxidase

Part number	**Designation**
UP99619A	UptiLight HRP blotting chemiluminescent substrate Contains reagent A (2x250ml, Luminol), and reagent B (2x15ml, oxydizer) Quantity sufficient for the detection of ca 80 miniblots (8x8cm)
UP99619D	UptiLight HRP blotting chemiluminescent substrate * Special Packaging * Contains reagent A (20x8ml, Luminol), and reagent B (15ml, oxydizer) Quantity sufficient for the detection of 20 miniblots (8x8cm)

Storage : +4°C, avoid direct light

Stability: 1 year from purchase date, according to 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, are probed with several reagents, the last step consisting of enzyme labeled reagent. Overcoming the performances (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 glow 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 ready and easy to use, and economical: UptiLight for HRP blots ensures optimal results, with lowest background and best detection signal, and allows multiple records by autoradiography. Uptima guaranties a stability of 1 year in the condition of lab use, with repeated changes of temperature from +4°C to room temperature when the stability of other commercial products are given for constant +4°C storage.

Scientific and Technical Information

- The sensitivity is very high: UptiLight was successfully used to detect as low as 1.0pg 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 lowest 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 (ferricyanure, cobalt, copper) or other compounds (hematin), -contaminated chemicals, may catalyse the luminol reaction.
- UptiLight has been used successfully with chemiluminescent detection scanners (Fuji (ray technology), Science technology (Chemimager), Berthold (Perkin Elmer))

Directions for Use

Use only clear recipients: used disposable test tubes for small volumes. If recipients should be used again (becher), wash them with suitable cleaning agent and rince well with distilled water. Traces of metals, immunoreagents, or detergents may affect the results.

The following protocol of blotting is given for a standard mini blot 8x8cm². The choice of temperature and duration of incubation, and the antibody and saturating buffers may be modified for special applications, 1/10th of saturant concentration may be added to probes incubation buffer. Ask Uptima for blot handling precautions (troubleshooting).

Protocol:

Preparation of the blot	Perform the blotting steps according to usual procedures. Take care of using immunological grade reagents *Western, Northern and Southern blotting:* separation of molecules (proteins, nucleic acids) by electrophoresis, then transfer onto nitrocellulose sheets *Dot blotting:* antigens deposited on spots Compatible blotting membranes: nitrocellulose, PVDF, nylon, Magna…	
Saturation	5% BSA, or 5% fatty free milk, Tween20® 0.1% , or SeaBlock (# UP40301A)	1 H +37°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%* *Primary antibody* *nucleic acid probe* *Peroxidase labeled probe*	1 H +37°C
Wash	1 time for 5 min with 20ml PBS+Tween20® 0.01% 2 times for 10 min with 20ml PBS+Tween20® 0.01%	
Final wash Substrate	2 times for 5 min with 20 ml of PBS; throw out well the wash Allow UptiLight reagent to reach room temperature, avoid direct light. Put 8ml of A reagent for 1 miniblot 8x8cm2 in a clean 10x10cm2 box Add 8 drops (ca 400ul) of reagent B, mix	Use immediately (or keep +4°C < 24H)
Incubation	Dry out the blot from PBS bath Put it in the bath of UptiLight mixture substrate, 1 min	1 min
Radiographic staining	Put it in a radiographic cassette, and cover with a clean plastic film	
	In a dark room: In a dark room, switch off the light Cover the blot with a radiographic film and expose 1min Put a new film to obtain a new record Stain the radiographic film in developing then fixating agents The time of further radiographic exposure can be adjusted for best results and multiple copies, from the previous results.	
	The blot can be kept for other types of analysis. Ask Uptima for possible striping and reprobing the blot.	

Other Information

Trouble Shooting

Problem	Causes	Answer
Background is to high, and homogenous	Antibody concentration is to high	dilute you primary and/or secondary antibody
	Buffer	Prepare fresh buffers , change the saturating agent
	Bad wash	Increase the duration of wash, remove better buffer before adding the fresh buffer
	Exposure	Reduce exposure time of the blot with the radiographic film
	Unsuitable membrane	Use an other type of membrane (NC)
Background is to high, but heterogenous	Traces	Take care of membrane handling (wear gloves to avoid skin contact, don't strip…)
	Dots and zones	Check the saturating agent is well dissolved; check that there is enough reagents solution to cover well the blot under constant agitation
No signal	Transfert	Check proteins are corectly transferred to the blot by a reversible staining (Ponceau); Put more protein if needed

For any question, please ask Uptima

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