

UptiLight One Spray

Chemiluminescent substrate for Western Blotting

Description

*The most convenient chemiluminescent substrate for peroxidase ever designed for Western blotting:
Just spray the one component, and expose !*

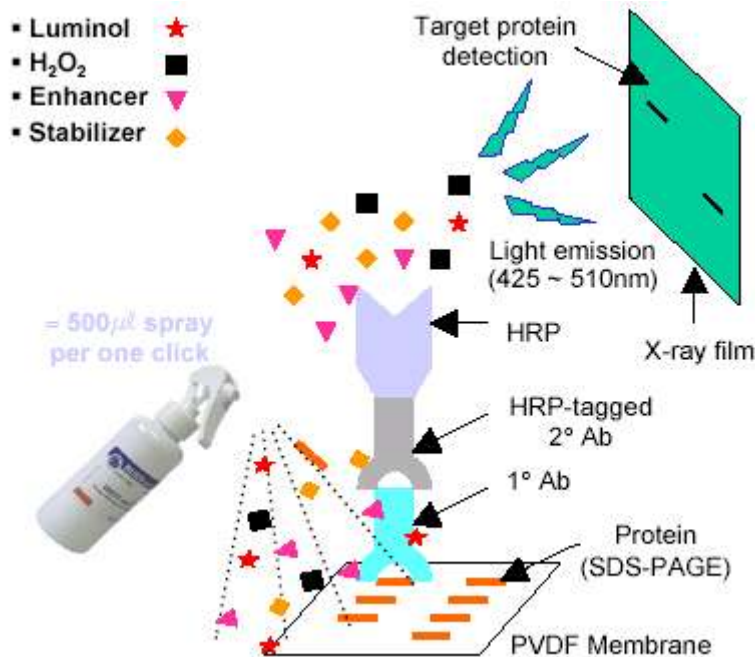
Catalog #: [BM4961](#), 1 kit (100ml, qsp 100 blots or 2500cm²)
Name: UptiLight One Spray WB chemiluminescent substrate

Storage: +4°C in dark room
Do not expose to heat or light.

Applications: immobilized peroxidase based technique (optimized for Western-blotting)

Benefits:

Spray type Rapid reaction time High Sensitivity Long duration time	→ Easiest to use: just spray, and expose blot to X-ray film → Specific detection achieved in less than 1-10minute → High contrast signal, detect as little as 1-2pg of antigen
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This sheet contains: [Directions for use](#) [Technical information](#)

Directions for use

Protocole 1:

For optimal results, it is essential to optimize the concentrations of both primary and secondary antibodies.

1/ Spray the UptiLight One Spray solution to give sufficient to cover the membranes

Note: The volume of one click is 500µl. 2 spray clicks are sufficient to cover a 25cm² membrane (i.e. 5cmx5cm)

2/ Incubate for 1 minute at room temperature without agitation

Note: The reaction for 1 minute is sufficient. Do not incubate more.

3/ Drain off excess detection reagent by holding the membrane vertically and touching the edge of the membrane against tissue paper.

Note: For less background, excess detection reagent on membrane has to be removed

4/ Wrap membranes in Saran Wrap and gently smooth out air pockets. Then place the blots, protein side up, in the film cassette.

Note: It is necessary to work quickly once the membrane has been exposed to the detection system.

5/ Switch off the lights and carefully place a sheet of X-Ray film on top of the membrane, close the cassette and expose for dozens of minutes.

Note: Do this in dark room using red shifted safelight.

Note: How long to continue exposure depends on the amount of target protein to detect on the membrane. Exposure from 30seconds to 5 minutes is usually sufficient to detect abundant proteins. Rare protein may require longer exposure up 30min.

Technical and Scientific Information

UptiLight™ One Spray is a sensitive and unique chemiluminescent substrate for peroxidase designed for un-rivalled ease of use in Western blotting (on component spray format). Beside its excellent sensitivity and stability, it's main advantage rely on its unrivalled ease of use, compared to any other chemiluminescent substrate that are liquid 2 components kits: just spray the reagent, provided in spray bottle, on you blot!

- Principle

UptiLight™ One Spray system is based on luminol based chemiluminescence generated by peroxidase activity. A strong light emission occurs at 425-510nm. The WB reagent is designed for detection of proteins (or other molecules) previously immobilized and probed by HRP labeled antibodies (or other ligand). Light is recorded by exposure to X-ray films, showing immunodetected specific protein bands. See the principle schema above.

- Applications

UptiLight™ One Spray is optimized for Western-blotting with horseradish peroxidase (HRP, POD) probes on PVDF or nitrocellulose membranes, but it may be applied to other immobilized peroxidase based technique as in-situ IHC.

- Sensitivity

UptiLight™ One Spray provides high sensitivity detection in immunoblotting, giving high contrast signal. Its high chemiluminescence signal and low background allow using very diluted I and II antibodies when probing blots. It is compatible with standard saturating agents.

- Ease of Use

UptiLight™ One Spray is quite different of all other western blot detection systems, that contain a substrate solution and an enhancer solution. UptiLight™ One Spray consists of one solution containing both substrate and enhancer, with proprietary additives, formulated in spray type container. The most remarkable characteristic is there is no mix step required, and operating is considerably simplified! Just spray the reagent! This results in gain of time, no need of measuring devices (tips, containers), less risk to contaminate the reagent, more reproducible working solution, and finally better and quicker results.

Contact your local distributor

interbiotech@interchim.com

Trouble shooting

Problem	Possible cause	Suggestions
No signal	Transfer	Check that transfer equipment is working properly Check protein transfer by staining the gel or membrane Check membrane nature
	Protein / Ab	Check extraction procedure do not alter protein (cleavage, not recognized by the I Ab). Increase protein deposited on the blot. check I Ab recognize protein.
Weak signal	Blotting procedure - Transfer	As above; check the transfer conditions
	Antibodies	Check the concentration of I and II Abs, and, provided there is no background, try lower combined dilutions. A signal test can be performed (a)
	Probing procedure - Washing	Check the washing buffer. Some buffer additives may remove bound low-affinity I Abs (concentration of detergent too high), or inhibit peroxidase (azide). Exposure time to radiographic film may have been too short.
High background	Antibody	The concentration of I and/or II may be too high
	Membrane	The blotting membrane was allowed to dry during some of the preparation or probing steps. Increase buffer volumes; improve agitation during incubations.
	Probing procedure - Saturation	The blotting membranes was not saturation properly avec the transfer step, or the saturating agent is not suitable to your application (I Ab). Try other blockers.
	Buffers contamination	Contamination interfering substances (i.e. hemoglobin) may be transferred to membrane from electrophoresis or blotting buffers and equipments Saturation, washing, dilution and probing buffers may also have been contaminated (bacteria,...)
	Probing procedure - Washing	The level of Tween20 was not sufficient
Artifacts	Blotting and probing procedures	The membrane was damaged or altered during on or several steps. Wear lab gloves to handle the membrane during transfer, saturation, probing; use a grip to handle the membrane (touch corners only). Filter the buffers (particulates may stick to the membrane).

(a)Signal test. Mix 10µl of peroxidase labeled probes (usually your II antibody) 1/1000 with 1ml of UptiLight™One Spray. A glow should be visible within seconds in dark room.

Other Information

For in vitro R&D use only NT-Comparison
Please contact Interchim for any other information NT-Technical tips

Please ask Interchim regarding associated products:

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| .Precise quantitation of ag before SDS-PAGE analysis: i.e. | BC Assay protein quantification kit | #40840A |
| .Reprobing UptiLight revealed blots: i.e. | Antibody stripping buffer | #L7710A |
| .Colorimetric or fluorescent protein staining in gel: | CooBlue | #G4562A and |
| | ProRed | #BD3631 |
| .Blotting Membranes: i.e. | TotaBlot™, Nytran™ membranes | |
| .Saturating agents: i.e. | SeaBlock™ (fish serum based), BSA 30% solutions,
BioBlock™ (milk based) | |
| .Buffers: i.e. PBT, TBS | | |
| .I and II Abs | | |

For any product, please search at <http://www.interchim.com/interchim/customers/articles.cfm>

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Contact your local distributor

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