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serving science through innovation

LiteAblot® EXTEND

Long Lasting Chemiluminescent Substrate

EMP013001 Sufficient for 1000 cm^2 of membrane	EMP013200 Sufficient for 200 cm^2 of membrane
Solution A - Luminol/Enhancer 50 ml amber bottle	Solution A - Luminol/Enhancer 10 ml amber bottle
Solution B - Peroxide Buffer 50 ml clear bottle	Solution B - Peroxide Buffer 10 ml clear bottle

For Research Use Only

Storage and Stability
One year at 2-8°C

Introduction
LiteAblot®EXTEND Long Lasting Chemiluminescent Substrate from EuroClone Life Sciences is a non-radioactive light emitting substrate for the detection of high femtograms amounts of immobilized specific antigens, conjugated directly or indirectly with horseradish peroxidase-labelled antibodies.

IMPORTANT NOTES
· Due to the extreme sensitivity of the system, optimization of both primary and secondary antibodies concentration is essential in order to get results with a high signal and low background.
· To achieve the highest signal-to-noise ratio it is strongly recommended to test different blocking reagents and empirically determine which is the most suitable for the western blot systems you are using.
· Addition of Tween®-20 to blocking and antibody dilution buffer reduces non specific signal.
· Throughout the procedure the blot membrane should never dry out: use always a proper amount of either blocking buffer, washing buffer, antibody solution and Substrate Working Solution. For optimal results perform incubation steps on a shaking platform.
· When handling film or detection reagents always wear powder-free gloves or use forceps.
· Do not use azide as a preservative for buffers as it is an inhibitor of horseradish peroxidase.
· Any equipment used for membrane treatment must be clean and free of foreign material. Traces of rust on either scissors, forceps or other metallic devices may cause high background
· LiteAblot®EXTEND working solution is stable for 24 hrs at RT. If not used immediately, it should be stored in an amber bottle to avoid prolonged exposure to intense light.

Protocol at a glance
Step 1: Protein Separation by Electrophoresis
Step 2: Protein Transfer to Membrane
Step 3: Blocking of Nonspecific Sites
Step 4: Membrane incubation with the primary antibody specific for the antigen to be detected
Step 5: Membrane Wash
Step 6: Membrane incubation with HRP-labeled secondary antibody
Step 7: Membrane Wash

Step 8: Membrane incubation with LiteAblot®EXTEND substrate for 5 minutes
Step 9: Membrane exposure to X-ray film or imaging instrument

Materials Required

Equipment Required

· Electrophoresis and electrotransfer apparatus for Western Blot
· Nitrocellulose membrane specifically intended for Western Blot
· Shaking platform
· Film Cassette
· X-ray film
· Developing and fixing reagents

Working Solutions

Dilution Buffer: Tris-buffered Saline (TBS)
Washing Buffer: TBS-Tween 0.1%
Blocking Buffer: Dissolve the proper blocking agent (Non Fat Dried Milk, BSA, etc) in TBS-Tween 0.1%.
Primary antibody: Dilute the antibody specific for the target protein in dilution buffer in order to get a 1 mg/ml stock solution. Use Blocking Buffer to prepare the antibody working dilution. The suggested working dilution range is between 1:1000 and 1:50.000 or 20 ng/ml -1 μg/ml.
HRP-secondary antibody: If the HRP-conjugated antibody is supplied as 1 mg/ml stock solution, the suggested working dilution range is beteween 1:50.000 and 1:250.000 or 4-20 ng/ml. Use Blocking Buffer to prepare the antibody working dilution.

Full-length Western Blot Protocol

Step 1 : Perform electrophoresis.
Step 2 : Transfer proteins to nitrocellulose membrane (PVDF or other membrane might be used as well but optimization may be required).
Step 3 : Incubate the membrane in blocking buffer for 1 hour on a shaking platform at room temperature. Membranes may be incubated in the blocking solution over night at 2-8°C if this is more convenient.
Step 4 : Remove blocking reagent. Incubate the membrane in diluted primary antibody for 1 hour on a shaking platform. **Please use the dilution factor suggested** in the Working Solution Section.
Step 5 : Wash the membrane with washing buffer. Perform at least 4-6 washes of 5 minutes on a shaking platform. Each wash must be done with fresh washing buffer at RT. To reduce background increase the number of washes and the buffer volume.
Step 6 : Rinse membrane and incubate in diluted HRP-labeled secondary antibody for 1 hour on a shaking platform. **Please use the dilution factor suggested** in the Working Solution Section.
Step 7 : Wash the membrane (see step 5).
Step 8 : Incubate the membrane in Working Solution for 5 minutes.
Prepare the LiteAblot®EXTEND Working Solution by mixing equal parts of the Luminol/Enhancer Solution and the Peroxide Buffer Solution. The total volume prepared must be sufficient to cover the membrane: consider a volume of 0.1 ml Working Solution per cm2 of membrane. The Working Solution is stable for 24 hours at room temperature. Intense light can harm the Working Solution: if it is not used immediately it should be stored protected from light. Typical laboratory light will not alter Working Solution.
Step 9 : Remove the membrane from Working Solution.
· Drain off excess detection reagent by holding the membrane vertically and touching edge on tissue paper.
· Place the membrane in a plastic membrane protector (plastic wrap may also be used) paying attention to remove excess of liquid and air bubbles.
· Place the wrapped membrane in the film cassette with the protein side facing up. Ensure that there is no free detection reagent in the film cassette: the film must remain dry during exposure.
· Switch off lights and place a sheet of film on top of the membrane, close the cassette and expose. Do not move the film during exposure to avoid any artifacts on the film.

· Expose for 60 seconds. Exposure time may be varied to achieve optimal results. If using a phosphor imaging system or a CCD camera longer exposure times may be necessary.
· Remove film and immediately place with a fresh piece of unexposed film and reclose film cassette. Light emission will go on for 24 hours and will decrease with time. Longer exposure times may be necessary as the blot ages.
Develop film using appropriate developing solutions and fixative.
Following LiteAblot®EXTEND detection you can store the wrapped membrane at 2-8°C. The membrane can then be stripped and reprobed several times.

Troubleshooting Guide

HIGH BACKGROUND	
Possible Cause	**Precautions/Remedies**
Antibody concentrations are too high	· Very high concentrations of both primary and/or secondary antibodies can cause high background. Decrease antibody dilutions.
Inadequate blocking	· Check that blocking agent solution has been made up correctly.
	· Include blocking agent in all detection reagent working solutions.
	· Try alternative blocking agents: 1-10% bovine serum albumin in TBS-T or PBS-T (freshly prepared) 0.5-3% gelatin in TBS-T or PBS-T (freshly prepared).
Inadequate washing	· Increase washing times and volumes of wash buffer.
	· Add Tween to reagents if not yet included.
	· Increase concentration of Tween in washing solution.
Contaminated buffers	· Ensure all buffers are freshly prepared and filtered.
Contaminated blot equipment	· Clean or replace all equipment.
Problems with membranes	· Check that the membranes are completely immersed in all solutions especially during washing, and that membranes hydrate thoroughly.
	· Use high quality nitrocellulose membranes.
	· Damage to the membrane can cause non-specific binding of the detection reagents. Handle blots carefully with gloved hands.
	· Use clean forceps to handle blots after washing.
Detection reagents	· Excess detection reagents in blots. Drain well by absorbing the excess on tissue paper before placing blots in film cassettes.
Over exposure	· Expose the film for a minimum period (an initial 15 seconds exposure may be all that is required). If exposure time is too short to be convenient, reduce antibody concentrations.
	· Leave blots in the cassette for 5–10 minutes before re-exposing to film.

WEAK OR NO SIGNAL	
Possible Cause	**Precautions/Remedies**
No transfer of proteins during Western Blot	· Stain membrane with protein stain to check for transfer efficiency.
	· Optimize gel acrylamide concentration, time for transfer and currently use molecular weight markers covering the molecular weight range expected to be blotted.
Insufficient quantities of antigen or antibody	· Increase amount of antigen.
	· Increase and optimize concentration and incubation times of primary antibody.
Poor binding of primary antibody	· Check that antigenicity is not detroyed by treatments required for electrophoresis (SDS, UREA, boiling etc). Check the antigen binding capacity of the antibody using a dot blot system.
Too much HRP in the system	· Excess of HRP can deplete the substrate and cause the signal to fade quickly. Dilute HRP-conjugate at least 10-fold.
Reduction of HRP or substrate activity	· Check that the detection reagents are being stored correctly and used as recommended.
	· Test the activity of the system: prepare a small volume (1 ml) of LiteAblot®EXTEND working solution and in the dark add 1µl of undiluted HRP-labeled antibody. Visible blue light should be produced.
NONSPECIFIC BANDS	
Possible Cause	**Precautions/Remedies**
Too much HRP in the system	Dilute HRP-conjugate at least 10-fold.
UNEVEN/SPOTTED BLOT	
Possible Cause	Precautions/Remedies
Improper blot technique	See above (no signal).
Unevenly hydrated membrane	Use new/fresh membranes.
	Make sure that membrane is fully covered and wet during incubations.
Fingerprints and / or keratin contamination	Avoid touching membrane. Use gloves and blunt forceps.
Bubble between the film and the membrane	Remove the bubbles before exposing blot to film.
WHITE (NEGATIVE) BANDS ON THE FILM	
Possible Cause	**Precautions/Remedies**
Too much HRP in the system	Dilute HRP-conjugate at least 10-fold.

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