

Western Lightning Ultra

Extreme Sensitivity Chemiluminescence Substrate

Catalog number	NEL111001EA	NEL112001EA	NEL113001EA
Enhanced luminol reagent Ultra	10 ml	55 ml	110 ml
Oxidizing reagent Ultra	10 ml	55 ml	110 ml
Membrane area	200 cm ²	1100 cm ²	2200 cm ²

Upon arrival both reagents should be stored at 2° - 30°C.

Western Lightning Ultra is a very sensitive chemiluminescence system for detection of horseradish peroxidase (HRP) on membranes, with maximum sensitivity in the low femtogram range. It incorporates a novel enhancer that intensifies a signal generated by peroxidase catalyzed oxidation of luminol. The substrate produces a signal at 425 nm for at least 8 hours, making it ideal for use with a wide variety of CCD imagers as well as Kodak BioMax® Light or X-OMAT Blue film. Membranes may be stripped and re-probed for detection of multiple targets.

Important Information

- Optimization of all system components is required for best results. The extreme sensitivity of Western Lightning Ultra typically requires reduced amounts of sample, antibodies and HRP as compared to standard substrates.
- These reagents have been formulated and are quality-controlled specifically for blotting applications. FOR LABORATORY USE ONLY.
- Western Lightning Ultra has been formulated for use on PVDF and nitrocellulose membranes.
- To achieve the maximum signal to noise ratio the primary and secondary antibodies should be optimized in a titration experiment.
- a. For primary antibodies, the typical dilution range from a 1 mg/ml stock is 1:5,000 to 1:100,000.
- b. For HRP conjugates, the typical dilution range from a 1 mg/ml stock is 1:100,000 to 1:500,000.
- Proper blocking and washing of membranes is critical for optimum results. The recommended blocking and washing conditions should be tried first and adjusted as necessary for a particular application.
- Avoid milk as a blocking reagent when using streptavidin as a detection reagent because it contains variable amounts of biotin.
- Phosphate buffers should not be used when phosphoproteins are being detected.
- Some components of the luminol or oxidizing reagents may precipitate if the product freezes during shipping. Mix moderately with a gentle swirling motion to ensure that all components are in solution.
- Do not use kit components beyond the expiration date. This date is printed on the kit label.
- Do not substitute reagents from other kits. Reagents have been optimized for performance with each kit lot. Dilution or other alteration of reagents may result in undesirable modifications of performance, such as loss of sensitivity.
- If membrane dries, wet with appropriate solvent
 - %2-PVDF: wet with methanol or 95% ethanol, rinse with water, then buffer
 - %2-Nitrocellulose: rinse with water, then buffer
- Prepare Western Lightning Ultra Chemiluminescence Working Solution by mixing equal parts of luminol reagent and oxidizing reagent. The solution is stable for 8 hours at room temperature. For best results, store Working Solution in an amber bottle and do not expose to intense light.
- Do not interchange bottle caps; this will lead to cross-contamination of reagents. Designate specific containers for specific reagents, and use clean pipettes or pipette tips for each reagent.
- Developing a first film after 30 seconds of exposure allows an estimation of the optimum exposure time to use. (Exposure time can vary from 30 seconds to 2 hours.)
- Except for film exposure and development, all steps can be performed outside the darkroom.

PROCEDURE SUMMARY

1. Membrane Preparation

- a. Separate proteins by electrophoresis and transfer to PolyScreen® PVDF or nitrocellulose membrane.
- b. Block non-specific binding sites by incubating the membrane in BLAST blocking buffer, 5% non-fat dry milk in PBST or TBST, or other blocking reagent as appropriate for at least one hour or overnight at 4°C with gentle agitation.
- c. Wash the membrane three times for 5 minutes with PBST or TBST.
- d. Dilute the primary antibody in blocking buffer or 1% BSA/PBST or TBST and incubate with the membrane for at least one hour or overnight at 4°C with gentle agitation.
- e. Wash the membrane with PBST or TBST once for 15 minutes, and then four times for 5 minutes each.
- f. Dilute the HRP-labeled second antibody in blocking buffer or 1% BSA/PBST or TBST and incubate with the membrane for at least one hour or overnight at 4°C with gentle agitation.
- g. Wash the membrane with PBST or TBST once for 15 minutes and then four times for 5 minutes each. The membrane may be left in buffer overnight at 4°C with gentle agitation.

2. Chemiluminescence Reagent Protocol

- a. Prepare the chemiluminescence reagent (0.1 ml of Chemiluminescence Reagent per cm² of membrane) by mixing equal volumes of the Enhanced Luminol Reagent and the Oxidizing Reagent.

REAGENT PREPARATION

10X Phosphate Buffered Saline (10X PBS) For 1 liter: NaH ₂ PO ₄ .H ₂ O 2.03 g Na ₂ HPO ₄ 11.49 g NaCl 85 g Adjust to pH to 7.3 to 7.5 with HCl. Storage: Room Temperature. Alternately, Dulbecco's Phosphate Buffered Saline without calcium chloride or magnesium chloride (available from commercial sources). Do not use phosphate buffers when detecting phosphoproteins.	10X Tris Buffered Saline (10X TBS) For 1 liter: Tris base 24.23 g NaCl 87 g Adjust to pH to 7.3 to 7.5 with HCl. Storage: Room Temperature.
10X PBS-TWEEN® 20 (10X PBST) For 1 liter: 10X PBS 995 ml TWEEN ® 20 5 ml A preservative such as thimerosal (1 g/L) may be added to prolong the life of the reagent. Do not use sodium azide because it inhibits HRP activity. Storage: Room Temperature.	10X TBS-TWEEN® 20 (10X TBST) For 1 liter: 10X TBS 995 ml TWEEN ® 20 5 ml A preservative such as thimerosal (1 g/L) may be added to prolong the life of the reagent. Do not use sodium azide because it inhibits HRP activity. Storage: Room Temperature.
1X PBST For 1 liter: 10X PBS-T 100 ml dH ₂ O 900 ml Storage: Room Temperature	1X TBST For 1 liter: 10X TBS-T 100 ml dH ₂ O 900 ml Storage: Room Temperature
Membrane Blocking Buffer (5% Non-Fat Dry Milk) For 100 ml: Carnation™ Instant Non-Fat Dry Milk 5 g 1X PBST or 1X TBST 100 ml If additional blocking capability is desired, this reagent may be supplemented with normal serum of the same type as the antibody. Casein or BSA may be substituted for the non-fat dry milk. This reagent should be made up fresh for every use..	
BLAST Blocking Buffer For 100 ml: BLAST Blocking Reagent (cat. no. FP1063) 1 g 1X PBST or 1X TBST 100 ml Add Blocking Reagent slowly to buffer with vigorous stirring. Stir the solution at room temperature for at least 1 hour. Then, heat the Blocking Buffer gradually (up to 60°C) with continuous stirring to dissolve the Blocking Reagent. The solution should be milky white with no precipitate evident. Aliquot and store at -20°C for long term use.	
Antibody Diluent (1% BSA) For 1 liter: 10X PBST or TBST 100 ml H ₂ O 800 ml BSA 10 g Adjust the pH to 7.4, add H ₂ O to 1 liter, and filter through a 0.22 µm membrane. Storage: 4°C	
Stripping Buffer 62.5 mM Tris-HCl pH 6.8 2% SDS 100 mM 2-mercaptoethanol	

- b. Incubate the membrane in the chemiluminescence reagent for one minute with gentle agitation.

3. Protein Visualization

- a. Remove excess chemiluminescence reagent by draining or blotting and place the membrane in a plastic sheet protector.
- b. **Film:** Expose to BioMax Light or X-OMAT Blue Autoradiography Film for 30 seconds. Develop the film and, if necessary, use the result to determine an optimum exposure.
Imager: Use optimum settings for chemiluminescence with luminol as recommended by manufacturer.

4. Stripping and reprobing (*Optional*)

- This protocol has been used on PolyScreen® and nitrocellulose membranes. Four successful reprobing have been carried out on both types of membranes.
- a. After the film or CCD exposure wash the membrane for 4 X 5 minutes in PBST or TBST.
 - b. Incubate the membrane for 30 minutes at 50°C in stripping buffer.
 - c. Wash the membrane for 6 X 5 minutes in PBST or TBST.
 - d. Incubate the membrane for 1 minute in Western Lightning Ultra. Expose to film or CCD for 1 minute to 1 hour to make sure that the original signal is removed.
 - e. Wash the membrane again for 4 X 5 minutes in PBST or TBST.
 - f. The membrane is now ready for reuse. Start at the blocking step (1b).

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Remedy
No signal/ weak signal	Poor transfer of proteins	<ul style="list-style-type: none"> • Check gel • Use Colored MW Markers. • Use correct pore size membrane for proteins >20 kD use a 0.45 m membrane <20 kD use a 0.22 m membrane
	Detergents, SDS, exhibit poor binding of low MW proteins	Remove SDS whenever possible
	Membrane preparation inadequate	Check proper membrane hydration Alcohol Alcohol-Water-Buffer
	Primary or secondary antibody concentration too low, too high or inactive	Titrate antibody conjugates for optimum concentrations or make up fresh
	Wrong blocking reagent	Test Blocking reagents with proteins for non affinity
	Azide inhibiting HRP activity	Use only azide-free reagents
	Chemiluminescence reagent improperly prepared	Add HRP conjugate to reagent and look for visible light in a darkroom
	Precipitation of components in luminol or oxidizing solutions because of freezing	Mix moderately to ensure that all components are in solution
Excess signal/Non Specific Binding	Antigen or antibody excess	Adjust concentrations by optimization experiments
High Background	Antigen or antibody excess	Adjust concentrations by optimization experiments
	Cross Reactivity of Blocking Reagent & Antibody	Test blocking buffers or use Tween-20 in Wash Buffer
	Overexposure to film	Shorter film exposure or let signal decay for 10-15 minutes and repeat exposure
	Membrane dried out during incubation	Use enough reagent to keep membrane wet
	Poor quality antibodies	Use good quality affinity purified antibodies
White Bands or "Antibands"	Blank bands on film caused by depletion of chemiluminescence substrate at sites of excess antigen and/or antibody	Reduce concentration of the secondary HRP labeled antibody
"Blotchy" Blot	Fingerprints, metal forceps, gloves	Use powder free gloves and avoid touching or folding the membranes
Speckled background	Blocking Reagent Secondary HRP conjugated Ab	Filter using 0.45 m aqueous filter Spin for 10-20 seconds, use supernatant

RELATED PRODUCTS

BLAST Blocking Reagent	5 g	FP1063
Multicolored Protein Markers	500 µl	NEL316001EA
PolyScreen PVDF Hybridization Transfer Membrane	1 (26.5 cm x 3.75 m) roll	NEF1002001PK
	50 (7 x 8.4 cm) sheets	NEF1003001PK
Protran® Nitrocellulose (0.2 m pore size)	1 (30 cm x 3 m) roll	NBA083C001EA
Protran® Nitrocellulose (0.45 m pore size)	1 (30 cm x 3 m) roll	NBA085C001EA
Anti-rabbit IgG (goat) HRP	1 mg, 1 mg/ml	NEF812001EA
Anti-mouse IgG (goat) HRP	1 mg, 1 mg/ml	NEF822001EA
Anti-human IgG (goat) HRP	1 mg, 1 mg/ml	NEF802001EA
Streptavidin-HRP		NEL750001EA
BioMax® Light-1 Autoradiography Film	13 x 18 cm (5 x 7 in.)	8689358001EA
X-OMAT Blue (XB) Film	13 x 18 cm (5 x 7 in.)	NEF586001EA

ADDITIONAL INFORMATION

Please visit www.perkinelmer.com/western for additional information including a complete product manual and related products for western blotting. Technical Support is available via email as follows.

In Europe:

techsupport.europe@perkinelmer.com

In U.S. and Rest of the World:

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REFERENCES

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Towbin, H., Staehelin, T. and Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *PNAS* 76:4340-4354 (1979).

This product is subject to US patents 7,855,287 and 7,803,573, assigned to Cyanagen srl, together with other equivalent granted patents and patent applications in other countries.

