Western Lightning Plus

Enhanced Chemiluminescence Substrate for Western Blotting

	Western Lightning *Plus* (standard sensitivity)			
Catalog number	NEL103E001EA	NEL103001EA	NEL104001EA	NEL105001EA
Enhanced luminol reagent	15 ml	65 ml	170 ml	340 ml (2 bottles)
Oxidizing reagent	15 ml	65 ml	170 ml	340 ml (2 bottles)
Membrane area	240 cm^2	1000 cm^2	2500 cm^2	5000 cm^2

Upon arrival both reagents should be stored at 2° - 8°C.

Western Lightning Plus is a non-radioactive light-emitting system designed to detect proteins immobilized on a membrane. The method provides sensitivity of 1 - 10 pg of protein. Optimal results are attained by using BioMax® Lightor X-OMAT Blue film, as well as appropriate imaging systems. Membranes may be stripped and re-probed.

Important Information

- These reagents have been formulated and are quality-controlled specifically to detect proteins in Western blots. FOR LABORATORY USE ONLY.
- Western Lightning Plus 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 suggested dilution range from a 1 mg/ml stock is 1:500 to 1:5,000 (or as recommended by manufacturer).
  b. For HRP conjugates, the suggested dilution range from a 1 mg/ml stock is 1:1,000 To 1:20,000
- 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.
- Phosphate buffers should not be used when phospho-proteins 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 the Chemiluminescence Reagent immediately before use. Prepare only enough for the membranes being processed. Discard any excess.
- 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 5% non-fat dry milk in PBST or TBST, BLAST blocking buffer, 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 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 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 (0.125 ml of Chemiluminescence Reagent per cm^2 of membrane) by mixing equal volumes of the Enhanced Luminol Reagent and the Oxidizing Reagent.
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 reprobings 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. 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).

REAGENT PREPARATION

10X Phosphate Buffered Saline (10X PBS)
For 1 liter: $NaH_2PO_4.H_2O$ 2.03 g; Na_2HPO_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_2O 900 ml
Storage: Room Temperature

1X TBST
For 1 liter: 10X TBS-T 100 ml; dHO 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_2O 800 ml; BSA 10 g
Adjust the pH to 7.4, add H_2O 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

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Remedy
No signal/ weak signal	Poor transfer of proteins	Check gel Use Colored MW Markers. Use correct pore size membrane for proteins >20 kD use a 0.45 µm membrane <20 kD use a 0.22 µm membrane
	Detergents, SDS, exhibit poor binding of low MW proteins	Remove SDS whenever possible
	Membrane preparation inadequate	Check proper membrane hydration 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 µm 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
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

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:
techsupport@perkinelmer.com

REFERENCES

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