Western Lightning Plus
Enhanced Chemiluminescence Substrate for Western Blotting

### 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 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:  
- NaH₂PO₄·H₂O: 2.03 g
- Na₂HPO₄: 11.49 g
- NaCl: 85 g

Adjust to pH to 7.3 to 7.5 with HCl.

Storage: Room Temperature.

#### 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.

#### 1X 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.

#### 1X PBST

For 1 liter:  
- 10X PBS-T: 100 ml
- dH₂O: 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 PBS 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 PBS or TBST: 100 ml
- BSA: 10 g

Adjust the pH to 7.4, add dH₂O 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

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Upon arrival both reagents should be stored at 2°C - 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® Light or 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.
- 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).
- 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 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 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.
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal/ weak signal</td>
<td>Poor transfer of proteins</td>
<td>Check gel.</td>
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<tr>
<td></td>
<td></td>
<td>Use Colored MW Markers.</td>
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<tr>
<td></td>
<td></td>
<td>Use correct pore size membrane for proteins</td>
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<tr>
<td></td>
<td>&gt; 20 kD use a 0.45 µm membrane</td>
<td>Use correct pore size membrane for proteins &lt;20 kD use a 0.22 µm membrane</td>
</tr>
<tr>
<td>Detergents, SDS, exhibit poor binding of low MW proteins</td>
<td></td>
<td>Remove SDS whenever possible</td>
</tr>
<tr>
<td>Membrane preparation inadequate</td>
<td></td>
<td>Check proper membrane hydration Alcohol-Water-Buffer</td>
</tr>
<tr>
<td>Primary or secondary antibody concentration too low, too high or inactive</td>
<td></td>
<td>Titrate antibody conjugates for optimum concentrations or make up fresh</td>
</tr>
<tr>
<td>Wrong blocking reagent</td>
<td></td>
<td>Test Blocking reagents with proteins for non affinity</td>
</tr>
<tr>
<td>Azide inhibiting HRP activity</td>
<td></td>
<td>Use only azide-free reagents</td>
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<tr>
<td>Chemiluminescence reagent improperly prepared</td>
<td></td>
<td>Add HRP conjugate to reagent and look for visible light in a darkroom</td>
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<tr>
<td>Precipitation of components in luminol or oxidizing solutions</td>
<td></td>
<td>Mix moderately to ensure that all components are in solution</td>
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<tr>
<td>because of freezing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excess signal/Non Specific Binding</td>
<td>Antigen or antibody excess</td>
<td>Adjust concentrations by optimization experiments</td>
</tr>
<tr>
<td>High Background</td>
<td>Antigen or antibody excess</td>
<td>Adjust concentrations by optimization experiments</td>
</tr>
<tr>
<td>Cross Reactivity of Blocking Reagent &amp; Antibody</td>
<td>Test blocking buffers or use Tween-20 in Wash Buffer</td>
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<tr>
<td>Overexposure to film</td>
<td>Shorter film exposure or let signal decay for 10-15 minutes and repeat exposure</td>
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<tr>
<td>Membrane dried out during incubation</td>
<td>Use enough reagent to keep membrane wet</td>
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<tr>
<td>Poor quality antibodies</td>
<td>Use good quality affinity purified antibodies</td>
<td></td>
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<tr>
<td>White Bands or “Antibands”</td>
<td>Blank bands on film caused by depletion of chemiluminescence substrate at sites of excess antigen and/or antibody</td>
<td>Reduce concentration of the secondary HRP labeled antibody</td>
</tr>
<tr>
<td>“Blotchy” Blot</td>
<td>Fingerprints, metal forceps, gloves</td>
<td>Use powder free gloves and avoid touching or folding the membranes</td>
</tr>
<tr>
<td>Speckled background</td>
<td>Blocking Reagent Secondary HRP conjugated Ab</td>
<td>Filter using 0.45 µm aqueous filter Spin for 10-20 seconds, use supernatant</td>
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</tbody>
</table>

**RELATED PRODUCTS**

<table>
<thead>
<tr>
<th>BLAST Blocking Reagent</th>
<th>5 g</th>
<th>FP1063</th>
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</thead>
<tbody>
<tr>
<td>Multicolored Protein Markers</td>
<td>500 µl</td>
<td>NEL316001EA</td>
</tr>
<tr>
<td>PolyScreen PVDF Hybridization Transfer Membrane</td>
<td>1 (26.5 cm x 3.75 m) roll</td>
<td>NEF1002001PK</td>
</tr>
<tr>
<td>Protran® Nitrocellulose (0.2 m pore size)</td>
<td>1 (30 cm x 3 m) roll</td>
<td>NBA083C001EA</td>
</tr>
<tr>
<td>Protran® Nitrocellulose (0.45 m pore size)</td>
<td>1 (30 cm x 3 m) roll</td>
<td>NBA085C001EA</td>
</tr>
<tr>
<td>Anti-rabbit IgG (goat) HRP</td>
<td>1 mg, 1 mg/ml</td>
<td>NEF812001EA</td>
</tr>
<tr>
<td>Anti-mouse IgG (goat) HRP</td>
<td>1 mg, 1 mg/ml</td>
<td>NEF822001EA</td>
</tr>
<tr>
<td>Anti-human IgG (goat) HRP</td>
<td>1 mg, 1 mg/ml</td>
<td>NEF802001EA</td>
</tr>
<tr>
<td>Streptavidin-HRP</td>
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<td>NEL750001EA</td>
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**ADDITIONAL INFORMATION**

Please visit [www.perkinelmer.com/western](http://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**

