### Introduction

Chemiluminescent detection uses an enzyme to catalyze a reaction that results in the generation of visible light. The horseradish peroxidase (HRP) chemiluminescent reaction is based on the catalyzed oxidation of luminol by peroxide. Oxidized luminol emits light as it decays to its ground state. This technique has the speed and safety of chromogenic detection methods, at higher sensitivity levels.

Immobilon Western HRP Substrate provides high sensitivity in western or dot/slot/spot blotting applications on both PVDF and nitrocellulose transfer membranes, and is compatible with all commonly used buffers and blocking reagents. Blots on PVDF membrane may be reprobed, allowing detection of multiple target proteins on the same blot.

The HRP substrate consists of Luminol Reagent and Peroxide Solution. Working HRP substrate is prepared by combining equal volumes of Luminol Reagent and Peroxide Solution. The HRP substrate produces a high intensity signal with low background for detection of both high and low abundance proteins.

For more detailed immunodetection protocols, troubleshooting and background information on western blotting, see the Millipore Protein Blotting Handbook (pub. no. TP001 available on-line at www.millipore.com/publications.nl/docs/wp001).

### Kit Contents

- **Kit No. WBKL S00 50**
  - Covers 500 cm² of membrane area
  - Luminol Reagent, 25 mL
  - Peroxide Solution, 25 mL

- **Kit No. WBKL S01 00**
  - Covers 1,000 cm² of membrane area
  - Luminol Reagent, 50 mL
  - Peroxide Solution, 50 mL

- **Kit No. WBKL S05 00**
  - Covers 5,000 cm² of membrane area
  - Luminol Reagent, 250 mL
  - Peroxide Solution, 250 mL

**Storage conditions**: 2-8 °C

### Materials Required for Western Blotting

- **Immobilon-P or Immobilon PSQ PVDF membrane; or other membranes designed for Western Blotting Applications**
- **X-ray film and developer reagents or chemiluminescence-compatible imaging systems**
- **Blotting Filter Paper**
- **Solution. Working HRP substrate is prepared by combining equal volumes of Luminol Reagent and Peroxide Solution. The HRP substrate produces a high intensity signal with low background for detection of both high and low abundance proteins.**

### Usage Guidelines

- **Due to the high sensitivity of the Immobilon Western HRP Substrate, lower amounts of antigen and higher dilutions of primary and secondary antibodies are recommended.**
- **Typical primary antibody dilutions are 1:1,000–1:10,000 and secondary antibody dilutions typically range from 1:200,000–1:400,000.**
- **If switching to Immobilon Western HRP Substrate from a lower sensitivity substrate, previous antibody dilution factors may need to be increased at least five-fold for the primary antibody and two- to five-fold for the secondary antibody to achieve the optimal signal-to-noise ratio.**
- **Optimization of blocking reagents and incubation times will improve results and should be determined experimentally.**
- **The high sensitivity of the Immobilon Western HRP Substrate may result in a significant reduction in required exposure time. An initial exposure time of 30 seconds is recommended.**
- **Use of blocking buffer to dilute antibodies may reduce background and increase sensitivity.**

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**Materials Required for Western Blotting, continued**

- **Wash buffer; Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05%–0.1% Tween®-20 surfactant.**
  - PBS: 10 mM sodium phosphate, 150 mM NaCl, pH 7.2
  - TBS: 10 mM Tris, 150 mM NaCl, pH 7.4
- **Blocking buffer: 1–5% (w/v) blocking agent (e.g., casein, BSA, or nonfat dry milk) in wash buffer.**
- **Primary antibody specific for the protein of interest, diluted in wash buffer or blocking buffer.**
- **HRP-conjugated secondary antibody, specific for primary antibody, diluted in wash buffer or blocking buffer.**
- **Shallow trays, large enough to hold the blot.**
- **Plastic wrap, plastic bag, transparency or sheet protector.**
- **X-ray film and developer reagents or chemiluminescence-compatible imaging systems.**

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**Technical Assistance**

For more information, contact the Millipore office nearest you.

In the U.S., call 1-800-MILLIPORE (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech.service page on our web site at www.millipore.com/techservice.

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**Standard Warranty**

Millipore Corporation (“Millipore”) warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. MILLIPOL MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.

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**Ordering Information**

Millipore offers Immobilon Western Chemiluminescent Substrates and a variety of PVDF membranes for western blotting applications. See the Technical Assistance section for information about contacting Millipore Corporation.

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Catalogue No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immobilon Western Chemiluminescent Substrates for Western Blotting Applications</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Western Blotting Protocol

Protein Transfer
1. Resolve the protein mixture on a 1-D or 2-D polyacrylamide gel.
2. Immune the gel in an appropriate transfer buffer and allow it to equilibrate for 10–15 minutes.
3. If working with a PDVF membrane: Wet the membrane in 100% methanol for 15 seconds, or until the membrane appearance changes uniformly from opaque to semitransparent.
5. Equilibrate the membrane for at least 5 minutes in the transfer buffer.
6. Soak filter paper in the transfer buffer for at least 30 seconds.
7. Assemble the transfer stack as shown below.
   **CAUTION:** To ensure an even transfer, remove air bubbles by carefully rolling a clean pipette over the surface of each layer in the stack. Avoid excessive pressure that can damage the gel and membrane.

Antibody Incubations
1. If PVDF membranes were dried after transfer, wet the blots in 100% methanol for 15 seconds. The blot will uniformly change from opaque to semitransparent.
   **NOTE:** Omit this step if using nitrocellulose membrane.
2. Rinse the blot with water and then place the blot in blocking buffer and incubate for 1 hour with gentle agitation at room temperature.
3. Prepare primary antibody solution by diluting the antibody in wash or blocking buffer. See Usage Guidelines for antibody dilutions.
4. Place the blot in the diluted primary antibody solution and incubate for 1 hour with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.
5. Wash the blot with fresh wash buffer a minimum of three times with gentle agitation for 5–10 minutes. Additional or longer washes may further reduce background.
7. Allow the HRP substrate to reach room temperature (+10 minutes). Protection from light is not required.
8. Place the blot on a clean plastic wrap, and remove any air bubbles. Ensure that the surface of the plastic wrap or sheet protector is dry and unwrinkled.
9. Expose the blot to a suitable X-ray film for an appropriate duration. Because of the high sensitivity of the Immobilon Western HRP Substrate, a shorter exposure time may be preferable.
   **NOTE:** The volumes of working HRP substrate needed for some common membrane sizes are indicated below:
   
   | Blot Size | Working HRP Substrate Required
   |-----------|---------------------------------|
   | 7 x 5.5 cm | 6 mL (3 mL luminol reagent + 3 mL peroxide solution)
   | 10 x 10 cm | 10 mL (5 mL luminol reagent + 5 mL peroxide solution)
   | 8.5 x 13.5 cm | 12 mL (6 mL luminol reagent + 6 mL peroxide solution)

Membrane Stripping (Only for PDVF Membranes)
A single blot on PDVF membrane can be sequentially probed by stripping the first antibody from the blot, and then incubating with a different primary antibody. This is especially useful for method optimization or when sample amount is limited. The stripping process disrupts the antibody-antigen interaction, usually by a combination of detergent and heat or by exposure to low pH. Please refer to the Millipore Protein Blotting Handbook (TP001) for detailed stripping protocols.

Troubleshooting
This section describes solutions to problems you may encounter with western blotting protocols. Further information on related protocols, troubleshooting and background information on western blotting, can be found in the “Protein Blotting Handbook” (pub. no. TP001) available on-line at www.millipore.com/publications/nd/tp001.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>High background</td>
<td>Insufficient washes</td>
<td>Increase wash buffer volumes and wash cycle repetitions. Pre-filter all solutions including the transfer buffer using 0.45 µm Millex® syringe filter units or Steriflip® filter units.</td>
</tr>
<tr>
<td>Poor quality blotting reagents or buffers</td>
<td>Use high grade reagents and Milli-Q water.</td>
<td></td>
</tr>
<tr>
<td>Cross-reactivity between blocking reagent and antibody</td>
<td>Use TWEEN-20 surfactant in the washing buffer or use different blocking agent. Do not use milk as a blocking agent when using avidin-biotin systems as milk contains endogenous biotin.</td>
<td></td>
</tr>
<tr>
<td>Membrane drying during incubation processes</td>
<td>Use sufficient volumes to cover entire surface of the membrane during incubation.</td>
<td></td>
</tr>
<tr>
<td>Poor quality antibodies</td>
<td>Use high quality affinity-purified antibodies.</td>
<td></td>
</tr>
<tr>
<td>Secondary (enzyme-conjugated) antibody concentration too high</td>
<td>Decrease the antibody concentration and/or reduce x-ray exposure time. See Usage Guidelines.</td>
<td></td>
</tr>
<tr>
<td>Proteins present in the membrane</td>
<td>Use TWEEN-20 (0.05%–0.1%) surfactant in the wash and detection solutions to minimize protein-protein interactions and increase the signal-to-noise ratio.</td>
<td></td>
</tr>
<tr>
<td>Excess detection reagents</td>
<td>Drain blots completely before exposure.</td>
<td></td>
</tr>
<tr>
<td>Film has been overexposed</td>
<td>Shorten exposure time. Initial exposure of 30 seconds is recommended.</td>
<td></td>
</tr>
<tr>
<td>Blocker concentration too low</td>
<td>Load more antigen on the gel.</td>
<td></td>
</tr>
<tr>
<td>Insufficient blocking reagent</td>
<td>Do not use milk in the blotting solutions.</td>
<td></td>
</tr>
<tr>
<td>Insufficient washing or blocking</td>
<td>The blotting solution may have an affinity for the protein of interest and obscure the protein from detection. Try a different blocking agent and/or reduce the amount or exposure time of the blocking solution.</td>
<td></td>
</tr>
<tr>
<td>Antigen is being washed off the membrane by Tween-20 surfactant</td>
<td>Reduce or eliminate the use of TWEEN-20 surfactant except for the wash step following membrane blocking. Tween-20 solution should be 0.05–0.1%.</td>
<td></td>
</tr>
<tr>
<td>Primary antibody was raised against native protein</td>
<td>Separate subunits under denaturing conditions or use antibody to denatured antigen.</td>
<td></td>
</tr>
<tr>
<td>Insufficient antibody concentration</td>
<td>Increase the concentration of the antibody used. Prepare fresh antibody working solutions.</td>
<td></td>
</tr>
<tr>
<td>Insufficient antibody reaction time</td>
<td>Increases the incubation time.</td>
<td></td>
</tr>
<tr>
<td>Outdated substrate</td>
<td>Prepare fresh working HRP substrate and store properly. Outdated substrate can reduce sensitivity.</td>
<td></td>
</tr>
<tr>
<td>Specified background</td>
<td>Agarose in the blocking solution</td>
<td>Filter blocking reagent solution through 0.45 µm Millex filter unit.</td>
</tr>
<tr>
<td>Non-specific bands</td>
<td>Agarose in HRP-conjugated secondary antibody</td>
<td>Filter secondary antibody solution through 0.45 µm Millex or Steriflip unit.</td>
</tr>
<tr>
<td>Primary antibody concentration too high</td>
<td>Increase primary antibody dilution.</td>
<td></td>
</tr>
<tr>
<td>Secondary antibody concentration too high</td>
<td>Increase secondary antibody dilution.</td>
<td></td>
</tr>
<tr>
<td>Antigen concentration too high</td>
<td>Decrease amount of protein loaded on the gel.</td>
<td></td>
</tr>
<tr>
<td>Insufficient HRP-conjugated antibody</td>
<td>Reduce concentration of secondary, HRP-conjugated antibody.</td>
<td></td>
</tr>
<tr>
<td>Poor detection of small proteins</td>
<td>Small proteins are masked by large blocking molecules such as BSA.</td>
<td>Consider using gelatin or a low molecular weight polycrylamide (PV/P) as blocking agents.</td>
</tr>
<tr>
<td>Saturants such as TWEEN-20 and Triton X-100 have to be minimized.</td>
<td>Avoid excessive incubation times with antibody and wash solutions.</td>
<td></td>
</tr>
<tr>
<td>Avoid excessive incubation times with antibody and wash solutions.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use ImmoBlon® membrane for small proteins (&lt; 20 kDa).</td>
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<td></td>
</tr>
<tr>
<td>Primary antibody specificity too low</td>
<td>Use a different antibody.</td>
<td></td>
</tr>
<tr>
<td>Fingerprint, fold marks or forceps impressions on the blot</td>
<td>Avoid touching membrane with bare hands or folding membrane; use gloves and blunt end forceps.</td>
<td></td>
</tr>
<tr>
<td>Filter pads in wet tank transfer assembly are not clean</td>
<td>Clean filter pads thoroughly to remove oil and wash in transfer buffer before assembling the transfer stack.</td>
<td></td>
</tr>
</tbody>
</table>
Western Blotting Protocol

Protein Transfer

1. Resolve the protein mixture on a 1-D or 2-D polyacryl- 

amide gel.

2. Immune the gel in an appropriate transfer buffer and 

allow it to equilibrate for 10–15 minutes.

3. If working with a PVDF membrane: Wet the membrane in 100% methanol for 15 seconds, or until the membrane appearance changes uniformly from opaque to semitranspar- 

ent.

4. If working with a nitrocellulose membrane: Proceed to step 4. Nitrocellulose membranes do not require pre- 

wetting.

5. Equilibrate the membrane for at least 5 minutes in the 

transfer buffer.

6. Soak fiber paper in the transfer buffer for at least 30 minutes.

7. Assemble the transfer stack as shown below.

CAUTION: To ensure an even transfer, remove air bubbles by carefully rolling a clean pipette over the surface of each layer in the stack. Avoid excessive pressure that can damage the gel and membrane.

Chemiluminescent Detection

1. To prepare working HRP substrate, mix equal volumes of 

luminol Reagent and Peroxide Solution in a clean con- 

tainer or test tube. Approximately 0.1 mL of working HRP 

substrate is required per cm² membrane area.

The volumes of working HRP substrate needed for some 

common membrane sizes are indicated below:

<table>
<thead>
<tr>
<th>Blot Size</th>
<th>Working HRP Substrate Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 × 5.5 cm</td>
<td>6 mL (0.3 mL luminol reagent + 3 mL peroxide solution)</td>
</tr>
<tr>
<td>10 × 10 cm</td>
<td>10 mL (0.5 mL luminol reagent + 5 mL peroxide solution)</td>
</tr>
<tr>
<td>8.5 × 13.5 cm</td>
<td>12 mL (0.6 mL luminol reagent + 6 mL peroxide solution)</td>
</tr>
</tbody>
</table>

2. Allow the HRP substrate to reach room temperature (~10 minutes). Protection from light is not required.

3. Place the blot protein side up in a clean container, and 

add the HRP substrate onto the blot.

4. Incubate the blot for 5 minutes at room temperature.

5. Drain the excess substrate.

6. Cover the blot with a clean plastic wrap or sheet protector.

7. Transfer proteins according to blottin apparatus manufac- 

turer's instructions.

8. Remove the blot from the transfer system and briefly rinse the membrane in Milli-Q water to remove gel debris. Proceed with protocol below.

If required, the PVDF membrane blot may be air dried and stored refrigerated for several months.

Antibody Incubations

1. If PVDF membranes were dried after transfer, wet the blots in 100% methanol for 15 seconds. The blot will uniformly changes from opaque to semitransparent.

FORM: This step if using nitrocellulose membrane.

2. Rinse the blot with water and then place the blot in 

blocking buffer and incubate for 1 hour with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.

3. Prepar primary antibody solution by diluting the antibody in wash or blocking buffer. See Usage Guidelines for antibody dilutions.

4. Place the blot in the diluted primary antibody solution and 

incubate for at least 1 hour with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.

5. Wash the blot with fresh wash buffer a minimum of three 

times with gentle agitation for 5–10 minutes. Additional or 

longer washes may further reduce background. Immob- 

ilon-P™ (0.2 µm) membrane may require additional 

washing due to its greater surface area.

Membrane Stripping (Only for PVDF Membranes)

A single blot on PVDF membrane can be sequentially probed by stripping the first antibody from the blot, and then incubat- 

ing with a different primary antibody. This is especially useful for method optimization or when sample amount is limited. The stripping process disrupts the antigen–antibody interaction, 

usually by a combination of detergent and heat or by exposure to low pH. Please refer to the Millipore Protein Blotting Handbook (TP001) for detailed stripping protocols.

Antibody Incubations, continued

6. Prepare HRP-conjugated secondary antibody solution by 

diluting the antibody in wash or blocking buffer. See Usage Guidelines for antibody dilutions.

7. Place the blot in the diluted HRP-conjugated secondary 

antibody solution, and incubate for 1 hour with gentle agitation. Ensure that the solution moves freely across the entire surface of the membrane.

8. Wash the blot with fresh wash buffer a minimum of three 

times with gentle agitation for 5–10 minutes. Additional or 

longer washes may further reduce background.

Troubleshooting

This section describes solutions to problems you may encounter with western blotting protocols. Further information on related 

protocols, troubleshooting and background information on western blotting, can be found in the “Protein Blotting Handbook” (pub. 

no. TP001) available on-line at www.millipore.com/publications/n/docs/tp001. 

Problem | Possible Cause | Solution
--- | --- | ---
High background | Insufficient washing | Increase wash buffer volumes and wash cycles rep. 
Pre-filter all solutions including the transfer buffer using 0.45 µm Millipore® syringe filter units or filter/float™ filter units.

Poor quality blotting reagents or buffers | Use high grade reagents and Milli-Q water.

Cross-reactivity between blocking reagent and 

antibody | Use Tween-20 surfactant in the washing buffer or use different blocking agent.

Do not use milk as a blocking agent when using anti-bovine systems as milk contains bovine serum.

Membrane drying during incubation processes | Use sufficient volumes to cover entire surface of the membrane during incubations.

Poor quality antibodies | Use high quality affinity-purified antibodies.

Secondary (antigen-conjugated) antibody concentration too high | Decrease the antibody concentration and/or reduce x-ray exposure time. See Usage Guidelines.

Protein-protein interactions | Use Tween-20 (0.05–0.1%) surfactant in the wash and detection solutions to minimize protein-protein interactions and increase the signal-to-noise ratio.

Excess detection reagents | Drain Membrane completely before exposure.

Film has been overexposed | Slightly enhance x-ray exposure time. Initial exposure of 30 seconds is recommended.

Membrane was not re-blocked after stripping of the initial detection. | The membrane has to be blocked to the appropriate blocking agent prior to re-probing of the blot.

Insufficient washing or blocking | Increase the concentration or volume of the blocking agent used to compensate for the increased surface area of the membrane. In addition, incubation times for both the wash and blocking steps may need to be extended.

Weak or no signal | Insufficient protein transfer | Optimize protein transfer. If necessary, then the blot to visualize protein and confirm complete transfer. See Protein Blotting Handbook (TP001) for recommendations.

Antigen concentration is too low | Load more antigen on the gel.

Antigen is being washed off the membrane by Tween-20 surfactant | Reduce or eliminate the use of Tween-20 surfactant except for the wash step following membrane blocking. Tween-20 surfactant should be 0.05–0.1%.

Primary antibody was raised against native antigen | Separate or eliminate an endogenous contaminant or use antibody to denature antigen.

Antigen incubation is too low | Increase incubation time of antibody to increase antibody signal.

Antigen is inactive | Multiple freeze-thaw or bacterial contamination of antibody solution can change antibody activity. Prepare fresh antibody working solutions.

Insufficient antibody reaction time | Increase the incubation time.

Excess detection reagents | Drain Membrane completely before exposure.

Outdated substrate | Prepare fresh working HRP substrate and store properly. Outdated substrate can reduce sensitivity.

Specified background | Aggregates in the blocking reagent | Filter blocking reagent solution through 0.45 µm Millex filter unit.

Primary antibody concentration too high | Increase primary antibody dilution.

Secondary antibody concentration too high | Increase secondary antibody dilution.

Antigen concentration too high | Decrease amount of protein loaded on the gel.

Bioactive proteins | Too much HRP-conjugated antibody | Reduce concentration of secondary, HRP-conjugated antibody.

Poor detection of small proteins | Small proteins are masked by large blocking molecules such as BSA | Consider using casin, gelatin or a low molecular weight polyvinylpyrrolidone (PVP) as blocking agents.

Sambucus such as Tween-20 and Triton X-100 may have to be minimized. Avoid excessive incubation times with antibody and wash solutions.

Use Immobilon-M™ membrane for small proteins (< 20 kDa).

Uneven blot | Use a different antibody.

Fingerprint, fold marks or forces imprints on the blot | Avoid touching membrane with bare hands or folding membrane; use gloves and blunt end forceps.

Bubble between the membrane and X-ray film | Check blot for bubbles before exposure to the X-ray film.

Air bubbles were trapped during transfer | Use a pipette or a stirring rod, gently roll out any trapped air bubbles while moving gel.

Fiber pads in wet tank transfer assembly are not clean | Clean fiber pads thoroughly to remove salts and soak in transfer buffer before assembling the transfer stack.
Introduction

Chemiemunescence detection uses an enzyme to catalyze a reaction that results in the generation of visible light. The horseradish peroxidase (HRP) chemiluminescent reaction is based on the catalyzed oxidation of luminal by peroxide. Oxidized luminal emits light as it decays to its ground state. This technique has the speed and safety of chromogenic detection methods, at higher sensitivity levels.

Immobilon Western HRP Substrate provides high sensitivity in western or dot/slot/spot blotting applications on both PVDF and nitrocellulose transfer membranes, and is compatible with all commonly used buffers and blocking reagents. Blots on PVDF membrane may be reprobed, allowing detection of multiple target proteins on the same blot.

The HRP substrate consists of Luminal Reagent and Peroxide Solution. Working HRP substrate is prepared by combining equal volumes of Luminal Reagent and Peroxide Solution. The HRP substrate produces a high intensity signal with low background for detection of both high and low abundance proteins.

For more detailed immunodetection protocols, troubleshooting and background information on western blotting, see the Millipore Protein Blotting Handbook (pub. no. TP001 available on-line at www.millipore.com/publications.nsf/docs/tp001).

Materials Required for Western Blotting

- Wash buffer: Phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) containing 0.05–0.1% Tween-20 surfactant.
- PBS: 10 mM sodium phosphate, 150 mM NaCl, pH 7.2
- TBS: 10 mM Tris, 150 mM NaCl, pH 7.4
- Blocking buffer: 1–5% (w/v) blocking agent (e.g., casein, BSA, or nonfat dry milk) in wash buffer

NOTE: Immobilon Western HRP Substrate is compatible with all x-ray films, plastic wrap, plastic bags, transparent or sheet protector.

Cut Sheet 8.5 × 13.5 cm 10 IPVH 081 30
15 × 15 cm 10 IPVH 091 20
10 × 10 cm 10 IPVH 101 00
8 × 8 cm 50 IPVH 078 50

Blotting Filter Paper

- Millipore, Milli-Q, and Steriflip are registered trademarks of Millipore Corporation.
- Trition is a registered trademark of Union Carbide Corporation.
- Tween is a trademark of ICI Americas Inc.

Usage Guidelines

Due to the high sensitivity of the Immobilon Western HRP Substrate, lower amounts of antigen and higher dilutions of primary and secondary antibodies are recommended. Typical primary antibody dilutions are 1:1,000–1:10,000 and secondary antibody dilutions typically range from 1:20,000–1:200,000.

Optimization of blocking reagents and incubation times may improve results and should be determined experimentally.

The high sensitivity of the Immobilon Western HRP Substrate may result in a significant reduction in required x-ray film exposure time. An initial exposure time of 30 seconds is recommended. Optimum exposure time should be determined for each antibody system.

Always wear gloves and use blunt tip forceps (such as Millipore cat. no. XX62 000 06) when handling the membrane to avoid contamination.

Do not use sodium azide, which inhibits HRP activity, in any buffers or reagents.

Use of blocking buffer to dilute antibodies may reduce background and increase sensitivity.

Cut Sheet 7 × 8.4 cm 100 IBFP 078 5C
10 × 10 cm 100 IBFP 030 10

PVDF Membranes

- HRP Substrate
- AP Substrate

Storage conditions:

- 2–8 °C

Shallow trays, large enough to hold the blot

For more information, contact the Millipore office nearest you.

Ordering Information

Millipore offers Immobilon Western Chemiluminescent Substrates and a variety of PVDF membranes for western blotting applications. See the Technical Assistance section for information about contacting Millipore Corporation.