Phototope®-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody

☑️ 50 assays

System Includes:
- Anti-mouse IgG, HRP-linked Antibody #7076
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent* and 20X Peroxide #7003

Background: Chemiluminescent detection systems have emerged as the best all-around method for detection of Western blots. They eliminate the hazards associated with radioactive materials and toxic chromogenic substrates. The speed and sensitivity of these methods are unequalled by traditional alternatives. Because results are generated on film, it is possible to record and store data permanently, and blots detected with chemiluminescent methods are easily stripped for subsequent reprobing with additional antibodies. Horseradish peroxidase (HRP) conjugated secondary antibodies are utilized in conjunction with specific chemiluminescent substrates to generate the light signal. Horseradish peroxidase-antibody conjugates have a very high turnover rate, giving good sensitivity with short reaction times.

Applications: This product has been optimized for use in chemiluminescent Western blotting applications.

Method Overview:
There are six basic steps in the Western blotting procedures with the Phototope®-HRP Western Blot Detection System.

1. Polyacrylamide Gel Electrophoresis of Proteins: Separate the protein samples and molecular weight standards by polyacrylamide gel electrophoresis.
2. Transfer: Transfer the protein to membrane by standard electroblotting.
3. Block Membrane: Block to saturate nonspecific binding sites on the membrane.
4. 1° Antibody: Incubate the membrane with the primary antibody.
5. 2° Antibody: Incubate the membrane with HRP-linked anti-rabbit IgG and HRP-linked anti-biotin antibodies.

Advantages of CST’s Phototope®-HRP Western Detection System
- Sensitivity: Detection of subpicogram amounts of protein is routine with good primary antisera.
- Speed: Less than 1 hour is required for the entire detection procedure. Exposure times are seconds to minutes for the Phototope-HRP System.
- Multiple Exposures: Light is emitted at a constant rate for several minutes, so you can perform multiple exposures to optimize signal intensity. Re-exposure at a future date is achieved by simply adding more reagent.
- Stability: A permanent hard-copy record is generated that will not fade or disintegrate over time.
- Quantitative: X-ray films can be scanned to quantitate band intensities.
- Versatility: Kits are available for rabbit and mouse primary antisera.
- Simultaneous Detection of biotinylated molecular weight standards.

Description: The Phototope®-HRP Western Blot Detection System is designed for the chemiluminescent detection of proteins in standard Western blotting applications. Proteins and biotinylated molecular weight markers (provided) are separated by SDS-PAGE and transferred onto membrane. Following incubation with your primary anti-serum, horseradish peroxidase (HRP) linked secondary antibody and HRP-linked anti-biotin antibody are bound and then allowed to react with LumiGLO® reagent. The light emitted by destabilized LumiGLO® reagent is subsequently captured on X-ray film.

Storage: Store kit at –20°C. Some kit components may be stored at 4°C as specified on their product labels.

Recommended Antibody Dilutions:
- Anti-mouse IgG, HRP-linked 1:2000
- Anti-biotin, HRP-linked 1:1000

For Research Use Only. Not For Use In Diagnostic Procedures.
An extremely important component of an optimized Western blot is choice of membrane. Since nonspecific binding can result in high background, we have compiled the following list to use as a guideline when selecting a membrane.

Membranes were tested according to the protocol described in the Phototope-HRP Western Blot Detection System manual. The blocking agent was 5% nonfat dry milk for all blots. Membranes ranked as “recommended” showed little or no background, “acceptable” membranes showed a low to intermediate level of background, and “not recommended” membranes showed background that obscured the signal. It is likely that more extensive blocking could reduce the observed background.

These are empirical data generated in a fair and consistent manner, which should reflect the performance of these membranes using our systems. Membranes tested were samples provided by each manufacturer. Cell Signaling Technology does not lot-test these membranes, nor do we warrant a particular membrane for any purpose.

### PVDF Membranes

<table>
<thead>
<tr>
<th>Recommended</th>
<th>Acceptable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pall (FluoroTrans W, 0.2 μm)</td>
<td>Millipore (0.45 μm)</td>
</tr>
<tr>
<td>Gelman (0.45 μm)</td>
<td>MSI (0.45 μm)</td>
</tr>
<tr>
<td>Dupont-NEN (0.45 μm)</td>
<td>ICN (0.45 μm)</td>
</tr>
<tr>
<td>BioRad (0.2 μm)</td>
<td>Tropix (0.45 μm)</td>
</tr>
</tbody>
</table>

BioRad (0.2 μm) and S&S (0.2 μm) are not recommended.

### Nitrocellulose Membranes

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<th>Acceptable</th>
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</thead>
<tbody>
<tr>
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<td>Gelman (0.2 μm)</td>
</tr>
<tr>
<td>S&amp;S (0.2 μm) supported</td>
<td>Millipore (0.2 μm)</td>
</tr>
<tr>
<td>S&amp;S (0.45 μm)</td>
<td>MSI (0.45 μm)</td>
</tr>
<tr>
<td>BioRad (0.2 μm)</td>
<td>Novex (0.45 μm)</td>
</tr>
<tr>
<td>Amersham (0.2 μm)</td>
<td></td>
</tr>
<tr>
<td>Sigma (0.2 μm)</td>
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</tr>
</tbody>
</table>

### Nylon Membranes

None of the nylon membranes tested gave acceptable results.
Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. 1X SDS Sample Buffer: 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. 10X Tris Buffered Saline (TBS): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 1X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. Wash Buffer: 1X TBS, 0.1% Tween-20 (TBS/T)
8. Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 1X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 μl Tween-20 (100%).
9. Pholotope®-HRP Western Blot Detection System #7072: Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
10. Prestained Protein Marker, Broad Range (Premixed Format) #7720
11. Biotinylated Protein Ladder Detection Pack #7727
12. Blotting Membrane: This protocol has been optimized for nitrocellulose membranes; PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 μl per well of 6-well plate or 500 μl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 μl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 μl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight marker (#7720, 10 μl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 μl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.
SECTION 5. Physical and chemical properties

Information on basic physical and chemical properties
- **Physical state**: Liquid
- **Appearance**: Clear
- **Color**: Light yellow
- **Odor**: None
- **Threshold**: No information available
- **pH**: No information available
- **Melting point/freezing point**: Initial boiling point and boiling point: No information available
- **Flash point**: No information available
- **Evaporation rate**: No information available
- **Flammability (solid, gas)**: No information available
- **Vapor pressure**: No information available
- **Vapor density**: No information available
- **Relative density**: No information available
- **Reactivity**: No information available
- **Stability**: Incompatible with strong oxidizing agents, strong bases, strong bases.

SECTION 10. Stability and reactivity

- **Reactivity**: No information available.
- **Chemical stability**: Stable under recommended storage conditions.
- **Possibility of Hazardous Reactions**: None under normal processing.
- **Possibility of Hazardous Polymerization**: None under normal processing.
- **Conditions to Avoid**: No information available.

SECTION 3. Composition/Information on ingredients

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS No</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>dimethyl sulfoxide</td>
<td>67-68-5</td>
<td>&lt;0.05</td>
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</table>

SECTION 4. First-aid measures

- **Eye contact**: Rinse thoroughly with plenty of water, also under the eyelids. Keep eye wide open while rinsing.
- **Skin contact**: Wash off immediately with plenty of water for at least 15 minutes. If symptoms persist, call a physician. Remove and exchange contaminated clothing before re-use. Wash off immediately, with plenty of water. Immediate medical attention is not required. Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes.
- **Inhalation**: Move to fresh air. If symptoms persist, call a physician. Immediate medical attention is not required. If INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing.
- **Ingestion**: Immediate medical attention is not required. Rinse mouth. Drink plenty of water. Do NOT induce vomiting. Clean mouth with water. Do not induce vomiting without medical advice.

Most important symptoms and effects, both acute and delayed

- **Carefully avoid**: No information available.
- **Indication of any immediate medical attention and special treatment needed**: Treat symptomatically.

Advice for emergency responders

- **General advice**: Immediate medical attention is not required. Show this safety data sheet to the doctor in attendance. If symptoms persist, call a physician.
- **Protection of fire-fighters**: Use personal protective equipment.

SECTION 9. Fire-fighting measures

- **Suitable Extinguishing Media**: Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.
- **Unsuitable Extinguishing Media**: CAUTION: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from the chemical

- **Precautionary Statement(s)**: Wear personal protective equipment. Use of water spray when fighting fire may be inefficient.
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Clean Water Act

Disposal information

The material is not subject to regulation as a hazardous material for shipping.

SECTION 13. Disposal considerations

Waste Disposal Methods

Dispose of in accordance with all applicable national environmental laws and regulations.

Disposal considerations

Do not empty into drains; dispose of this material and its container in a safe way.

SECTION 14. Transport Information

This material is not subject to regulation as a hazardous material for shipping.

SECTION 15. Regulatory Information

Port: American Inventory Listing

Chemical Name TSCA 8(b) TSCA 12(b) DSL NDSL

dimethyl sulfoxide Listed Not Listed Listed Not Listed

Canada Workplace Hazardous Materials Information System (WHMIS) Classification:

Class D2B - Toxic Material at ≥ 1%

SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

SARA 311/312 Hazard Categories

Acute Health Hazard

Chronic Health Hazard

Fire Hazard

No

No

No

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Incompatible Materials

Strong oxidizing agents, Acid chlorides, Acid anhydrides.

Hazardous Decomposition Products

None known based on information supplied. Thermal decomposition can lead to release of irritating gases and vapors: Carbon oxides (COx), Sulfur oxides.

SECTION 11. Toxicological Information

Information on likely routes of exposure

Inhalation

Irritation of vapors in high concentration may cause irritation of respiratory system.

Eye contact

Expected to be an irritant based on components. Components of this product may be absorbed into the body through the skin.

Skin contact

Expected to be an irritant based on components. Components of this product may be absorbed into the body through the skin.

Ingestion

May be harmful if swallowed.

Information on toxicological effects

This material should only be handled by, or under the close supervision of, those properly qualified in the handling and use of potentially hazardous chemicals. It should be borne in mind that the toxicological and physiological properties of this compound is not well defined.

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Symptoms

No information available.

Skin corrosion/irritation

Irritating to skin.

Serious eye damage/eye irritation

Irritating to eyes.

Sensitization

No information available.

Mutagenic effects

No information available.

Carcinogenicity

No component of this product present at levels greater than or equal to 0.1% is identifiable as probable, possible or confirmed carcinogen by IARC, ACGIH, NTP, or CSIRA.

Reproductive toxicity

No information available.

STOT - single exposure

No information available.

STOT - repeated exposure

No information available.

Chronic Toxicity

Acute exposure: No information available.

Neurological effects

No information available.

Aspiration Hazard

No information available.

Other information

May accelerate skin absorption of other materials. Special attention needed when toxic materials are present in dimethyl sulfoxide because of enhanced skin absorption.

SECTION 12. Ecological Information

Ecotoxicity

Product does not present an aquatic toxicity hazard based on known or supplied information.

Chemical Name Toxicity to algae Toxicity to fish Toxicity to daphnia and other aquatic invertebrates

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Sudden Release of Pressure Hazard

No

Reactive Hazard

No

Clean Water Act

This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 394).

CERCLA

This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 302).

There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

California Proposition 65

This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

This product contains the following U.S. State Right to Know chemicals:

Chemical Name New Jersey Massachusetts Pennsylvania

dimethyl sulfoxide Listed Not Listed Listed

U.S. FIFRA Label Information

This product does not contain any substances regulated as pesticides.

U.S. Commerce Department - Export Administration Regulations Information

This product does not contain any substances regulated under the Chemical Weapons Convention (CWC).

U.S. Drug Enforcement Administration Information

This product does not contain any substances regulated under the DEA.

SECTION 16. Other Information

Issuing Date: 2015-01-09

Revision Date: 2015-01-09

Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet