

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

- ✓ Detection for 5,000 cm<sup>2</sup> of membrane (fifty 10 cm X 10 cm blots)

rev. 04/21/08

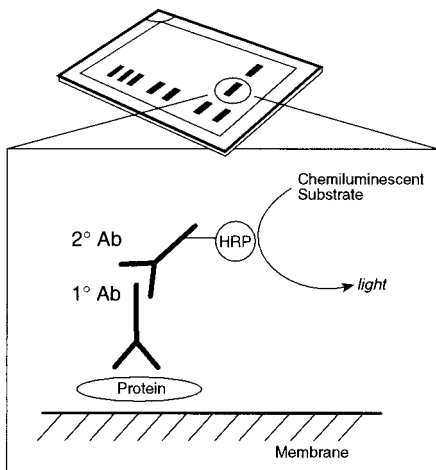
- Orders** ■ 877-616-CELL (2355)  
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

## System Includes:

- Anti-mouse IgG, HRP-linked Antibody #7076
- Anti-biotin, HRP-linked Antibody #7075
- 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.



After the primary antibody is bound to the target protein, a complex with HRP-linked secondary antibody is formed. The LumiGLO®\* is added and emits light during enzyme catalyzed decomposition.

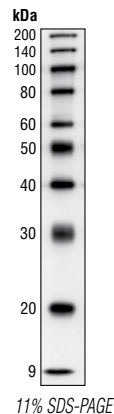
**Description:** The Phototope®-HRP Western Blot Detection System is designed for the chemiluminescent detection of proteins in standard Western blotting applications. Proteins and biotinylated molecular weight markers (provided) are separated by SDS-PAGE and transferred onto membrane. Following incubation 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.

**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.
- 6. Chemiluminescent Detection:** Add LumiGLO® Reagent and capture the emitted light 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

## 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

\*LumiGLO® is a trademark of Kirkegaard & Perry Laboratories (KPL). Avoid repeated exposure to skin (see MSDS on our website or request from CST or KPL).

Phototope® is a trademark of Cell Signaling Technology, Inc. Milli-Q™ is a trademark of Millipore.

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	
Recommended	Acceptable
Pall (FluoroTrans W, 0.2 µm)	Millipore (0.45 µm)
Gelman (0.45 µm)	MSI (0.45 µm)
Dupont-NEN (0.45 µm)	Novex (0.45 µm)
	ICN (0.45 µm)
	Tropix (0.45 µm)
BioRad (0.2 µm) and S&S (0.2 µm) are not recommended.	
Nitrocellulose Membranes	
Recommended	Acceptable
S&S (0.2 µm)	Gelman (0.2 µm)
S&S (0.2 µm) supported	Millipore (0.2 µm)
S&S (0.45 µm)	MSI (0.45 µm)
BioRad (0.2 µm)	Novex (0.45 µm)
Amersham (0.2 µm)	
Sigma (0.2 µm)	
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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-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.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- 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.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- 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.

- 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.

# Material Safety Data Sheet (MSDS) for LumiGLO® Reagent and Peroxide

Orders ■ 877-616-CELL (2355) orders@cellsignal.com

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Web ■ www.cellsignal.com

## I. Identification

**Product name:** 20X LumiGLO® Reagent and Peroxide

**CAS number:** N/A

**Catalog number:** 7003

## II. Physical Data

**Appearance:** Clear to faint yellow colored solution.

**Melting or Freezing point:** <0°C/32°F (water)

**Boiling Point:** >100°C/212°F (water)

**Solubility in water:** Dilutable

## III. Ingredients

**20X LumiGLO®:** Contains ≤20.0% weight % Dimethylsulfoxide (CAS number 67-68-5). This product is a mixture that may contain one or more hazardous chemicals. The hazardous ingredients listed above are only those as required by 29 CFR 1910. 1200 g 2.C1.

**Peroxide:** Reagent is not considered to be a hazardous product. It contains less than 1.0% hazardous chemical and less than 0.1% carcinogenic chemical.

## IV. Fire and Explosion Hazard Data

**Extinguishing media:** CO<sub>2</sub>, dry chemical.

**Special fire fighting procedures:** If involved in fire, don NIOSH/MSHA-approved self-contained breathing apparatus, flame/chemical resistant.

**Unusual fire and explosion hazards:** May emit toxic fumes under fire conditions.

## V. Health Hazard Data

**Threshold Limit Value (TLV) and source:** Data not available.

**Acute effects of overexposure:** To the best of our knowledge, the chemical, physical and toxicological properties have not been thoroughly investigated.

**Swallowing:** May be harmful if swallowed.

**Skin absorption:** May be harmful if absorbed through the skin.

**Inhalation:** May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.

**Skin contact:** May be harmful.

**Eye contact:** May cause eye irritation.

**Chronic effects of overexposure:** May be harmful.

### Emergency and First Aid Procedures

**Swallowing**—Wash out mouth with water, provided person is conscious. Call a physician.

**Skin**—In case of contact, immediately wash skin with soap and copious amounts of water.

**Inhalation**—If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

**Eyes**—In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating eyelids with fingers. Consult a physician.

## VI. Reactivity Data

**Stability/Conditions to avoid:** Not reactive as far as is known.

**Incompatibility/Materials to avoid:** Strong oxidizing agents, acid chlorides, acid anhydrides.

**Combustion/Decomposition products:** Carbon monoxide, carbon dioxide, sulfur oxides.

**Hazardous polymerization:** Not susceptible to polymerization.

## VII. Spill or Leak Procedures

**Steps to be taken if material is spilled or released:** Wear self-contained breathing apparatus, rubber boots and rubber gloves. Use Vermiculite or another suitable absorbent to clean up spill, place in a suitable closed container for disposal, then wash down spill site.

**Waste disposal method:** Dissolve the material in a combustible solvent and burn in an EPA-licensed chemical incinerator equipped with an after-burner and scrubber.

## VIII. Special Protection Information

**Respiratory protection:** Avoid inhalation. Use NIOSH/MSHA-approved respirator.

**Ventilation:** Use mechanical exhaust.

**Protective equipment:** Wear suitable protective clothing, chemical resistant gloves and lab safety glasses.

## IX. Special Precautions

**Handling and storage:** Store at 4°C.

**Precautions to be taken in handling and storage:** This compound is sold only for research use by personnel familiar with the toxicology of organic chemicals and who are well trained in good laboratory habits, such as avoiding spills, keeping hands clean at all times and not rubbing eyes with hands while working in the laboratory.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.

Revised: April 2001

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