

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

✓ 50 assays

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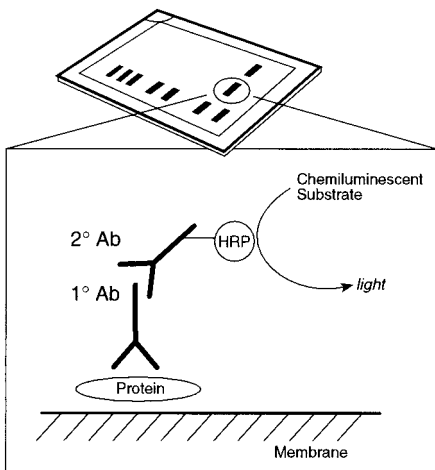
rev. 01/19/12

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

System Includes:

- Anti-mouse IgG, HRP-linked Antibody #7076
- Anti-biotin, HRP-linked (D5A7) Rabbit mAb #5571
- 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.

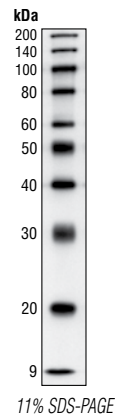
Description: The Phototope®-HRP Western Blot Detection System is designed for the chemiluminescent detection of proteins in standard Western blotting applications. Proteins and biotinylated molecular weight markers (provided) are separated by SDS-PAGE and transferred onto membrane. Following incubation 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 cm²) 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 20X LumiGLO® and 20X Peroxide



I. Identification:

Product name: 20X LumiGLO® and 20X Peroxide
Product Catalog: 7003
CAS number: None
Manufacturer Supplier: Cell Signaling Technology
 3 Trask Lane
 Danvers, MA 01923 USA
 1-978-867-2300 TEL
 1-978-867-2400 FAX
 1-978-578-6737 Emergency TEL

II. Composition/Information on Ingredients:

Hazardous Reagent:	Percent	CAS#
Dimethyl sulfoxide	≤20%	67-68-5

This product is For Research Use Only. According to 29 CFR 1910.1200(d), mixtures with hazardous ingredients at less than <1% and carcinogens at less than <0.1% are considered non-hazardous.

III. Hazard Identification:

CAUTION: This product is not for use in humans. It is intended for research purposes only. To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.

Emergency Overview: Irritant. Irritating to eyes, respiratory system, skin.

Potential Health Effects:

Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.

Eye Contact: May cause eye irritation.

Skin Contact: May be harmful if absorbed through skin. Prolonged or repeated contact may cause skin irritation.

Ingestion: May be harmful if swallowed.

IV. First Aid Measures:

Inhalation: Remove to fresh air. If breathing is difficult, get medical attention.

Ingestion: If person is conscious, wash out mouth with water. Get medical attention.

Skin exposure: Wash skin with soap and water. If irritation develops or persists, get medical attention.

Eye exposure: Immediately flush eyes water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: N/A

Autoignition Temperature: N/A

Explosion: N/A

Fire extinguishing media: water spray, dry chemical, alcohol foam, or carbon dioxide.

Firefighting: wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes. May emit toxic fumes under fire conditions.

VI. Accidental Release Measures:

Wear appropriate personal protective equipment as indicated in Section VIII. Absorb liquid with an absorbent material. Transfer contaminated absorbent to a closed chemical waste container for disposal. Wash spill site after material has been picked up for disposal.

VII. Handling And Storage:

Store at 4°C in tightly closed container.

Avoid inhalation of vapor or mist. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling. Avoid prolonged or repeated exposure.

VIII. Exposure Controls/Personal:

Ventilation System: a system of local and/or general exhaust is recommended.

Skin Protection: wear compatible chemical resistant gloves and protective clothing.

Eye protection: wear protective safety glasses or chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

IX. Physical And Chemical Properties:

Appearance:	clear faint yellow colored liquid
Odor:	data not available
pH:	data not available
Boiling Point:	>100°C/212°F (water)
Melting or Freezing Point:	<0°C/32°F (water)
Flash Point:	data not available
Volatile Organic Compounds (VOC):	data not available
Autoignition temp.	data not available
Solubility (water)	miscible in water

X. Stability and Reactivity:

Stability: Stable under normal conditions.

Conditions to avoid: strong oxidizing agents, strong acids, strong bases.

Hazardous Decomposition: carbon monoxide, carbon dioxide.

Hazardous polymerization: will not occur.

XI. Toxicological Information:

Acute toxicity: data not available. Chronic exposure: data not available

Potential Health Effects:

Inhalation: May be harmful if inhaled. Causes respiratory tract irritation.

Skin: May be harmful if absorbed through skin. Causes skin irritation.

Eyes: Causes eye irritation.

Ingestion: Harmful if swallowed.

Toxicity Data on Hazardous ingredient Dimethyl Sulfoxide, CAS#67-68-5
 RTECS: PV6210000
 LD50 Oral rat 14,500 mg/kg
 LC50 Inhalation rat 4 h 40250 ppm
 LD50 Dermal rabbit > 5,000 mg/kg

XII. Ecological Information:

No data available.

XIII. Disposal Considerations: Dispose of in accordance with federal, state and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material.

XIV. Transport Information:

D.O.T. Proper Shipping Name: None. This substance is considered non-hazardous for transport.

IATA Proper Shipping Name: None. This substance is considered non-hazardous for air transport.

XV. Regulatory Information:

EU: Not classified

OSHA: Ingredient Dimethyl Sulfoxide, CAS#67-68-5: Combustible Liquid, Target Organ Effect

Canadian DSL: Listed: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

SARA 302, 313 Ingredients Not Listed.

SARA 311/312: Ingredient Dimethyl Sulfoxide, CAS#67-68-5: Fire Hazard, Chronic Health Hazard.

Massachusetts Right To Know: Ingredients Not Listed.

Pennsylvania Right To Know: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

New Jersey Right To Know: Ingredient Dimethyl Sulfoxide, CAS#67-68-5

California Prop. 65: Ingredients Not Listed.

XVI. Other Information:

This product is for research use only and is not intended for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. The above information is believed to be accurate but is not necessarily all-inclusive and shall be used only as a guide. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.