SANTA CRUZ BIOTECHNOLOGY, INC.

Western Blotting Luminol Reagent: sc-2048

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

PRODUCT

Santa Cruz Biotechnology offers Western Blotting Luminol Reagent for enhanced chemiluminescence detection of Western blots. Luminol should be used in conjunction with horseradish peroxidase (HRP) conjugated secondary antibodies. One Luminol package contains 125 ml each Solution A and Solution B, which combine to form sufficient reagent for a 2,000 cm^2 membrane area.

PROCEDURE

- Perform Western blot onto nitrocellulose membrane according to Santa Cruz Biotechnology Research Applications or according to standard protocols. Probe membrane with primary antibody followed by HRP-conjugated secondary antibody. Secondary antibodies are available from Santa Cruz Biotechnology in a conventional format (catalog numbers sc-2004, sc-2005, sc-2006 and sc-2020) or in a format compatible with Santa Cruz Biotechnology Cruz Marker molecular weight standards (catalog numbers sc-2030, sc-2031, sc-2032 and sc-2033).
- Wash membrane three times for 5 minutes each with 1x TBS, 0.05% Tween-20. Wash once for 5 minutes with 1x TBS [1x Tris buffered saline (TBS): 10 mM Tris-HCl, pH 8.0; 150 mM NaCl].
- Mix equal quantities of Luminol Reagent Solution A and Solution B by inversion in a screw cap vial. Use 0.125 ml/cm2 total volume.
- Pour off TBS wash from the nitrocellulose membrane. Add the mixed Luminol Reagent Solutions to the membrane, protein side facing up. Incubate for 1 minute at room temperature.
- Pour off excess Luminol Reagent, or lift membrane with tweezers to drain excess Reagent and lightly dab the edge of the membrane with a chemwipe to remove excess solution. Use caution not to wipe or smear the membrane surface.
- Tightly cover the membrane, protein side facing up, with a single layer of plastic wrap. Be careful to prevent the formation of wrinkles or bubbles between the membrane and the plastic wrap.
- Carefully tape the plastic wrapped membrane, protein side facing up, to the paper backing. If desired, label membrane by applying phosphorescent tape to the outside of the plastic wrap. Place in the film cassette.
- In a darkroom, expose membrane to film for 1 minute and develop according to standard protocols for autoradiogram. Exposure period may be increased as necessary.

STORAGE

Luminol Reagent Solutions are stable at room temperature for one hour. Store at 2–8° C for longer periods.

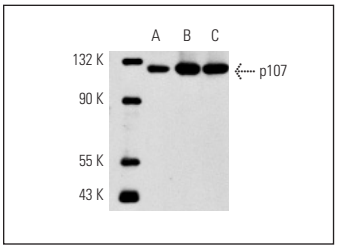
RESEARCH USE

For research use only, not for use in diagnostic procedures.

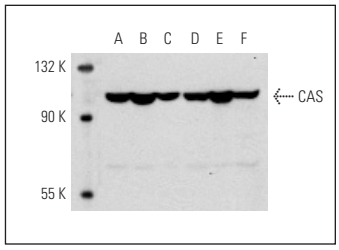
NOTES

- Have all materials ready before beginning the procedure. The following materials are needed: plastic wrap (enough to cover the blot), scissors, tweezers, tape, several pieces of film, film cassette, paper backing (size of film cassette) and a screw cap vial.
- Solutions contain chemicals that may bleach skin or clothing. Always wear gloves, protective clothing and eyewear to avoid solution contact.
- Nitrocellulose membrane should be handled with tweezers or gloved hands only.
- Work quickly and carefully as maximal detection and light emission due to enzymatic reaction occur during the first hour.
- If high backgrounds are observed following a 1 minute exposure of membrane to film, allow membrane to sit in the dark for 20–30 minutes at room temperature, followed by an additional 1 minute exposure of membrane to film. Alternatively, repeat the Western blot with a higher dilution of secondary antibody (up to 1:20,000).

DATA



p107 (C-18): sc-318-G. Western blot analysis of p107 expression in C32 (**A**), MM-142 (**B**) and KNRK (**C**) whole cell lysates.



CAS (N-19): sc-1709. Western blot analysis of CAS expression in NIH/3T3 (**A**), RAW 264.7 (**B**) and MM-142 (**C**) nuclear extracts and EOC 20 (**D**), F9 (**E**) and c4 (**F**) whole cell lysates.

SELECT PRODUCT CITATIONS

1. Paronetto, M.P., et al. 2004. Expression of a truncated form of the c-Kit tyrosine kinase receptor and activation of Src kinase in human prostatic cancer. Am. J. Pathology 164: 1243-1251.
2. Kowalski, M.P, et al. 2004. Localization of cystic fibrosis transmembrane conductance regulator to lipid rafts of epithelial cells is required for Pseudomonas aeruginosa-induced cellular activation. J. Immunology 172: 418-425.
3. Costamagna, E., et al. 2004. The functional interaction between the paired domain transcription factor Pax8 and Smad3 is involved in the TGF-β repression of the sodium iodide symporter gene. J. Biol. Chem. 279: 3439-3446.
4. Donev, R., et al. 2003. Recruitment of heterogeneous nuclear ribonucleoprotein A1 *in vivo* to the LMP/TAP region of the major histocompatibility complex. J. Biol. Chem. 278: 5214-5226.
5. Liu, J., et al. 2002. Aberrant production of IL-12 by macrophages from several autoimmune-prone mouse strains is characterized by intrinsic and unique patterns of NFκB expression and binding to the IL-12 p40 promoter. J. Immunology 169: 581-586.

SANTA CRUZ BIOTECHNOLOGY, INC.

Material Safety Data Sheet
Western Blotting Luminol Reagent: sc-2048

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CHEMICAL IDENTIFICATION

DESCRIPTION: Santa Cruz Biotechnology Luminol Reagent is a non-radioactive, light-emitting system for detecting proteins on a membrane. Horse radish peroxidase mediates oxidation of luminol in the presence of hydrogen peroxide and this reaction produces an iridescent light.

FORMULATION: 2 bottles; Solution A and Solution B.

USE: Incubate the membrane with equal volumes of solutions A and B for one minute. Remove the membrane from the substrate solution and drain the excess liquid. Cover the membrane with a transparent wrap. Immediately measure light emission using a camera luminometer or a film.

STORAGE AND STABILITY: Stable for 12 months at 4° C from date of shipment. Store in the dark.

COMPOSITION/INFORMATION ON INGREDIENTS

Ingredient Name: Dimethylsulfoxide
CAS: 67-68-5
Molecular Weight: 78.13
Molecular Formula: C_2H_6OS

Ingredient Name: Luminol
CAS: 521-31-3
Molecular Weight: 177.16
Molecular Formula: $C_8H_7N_3O_2$

Ingredient Name: p-Coumaric acid
CAS: 501-98-4
Molecular Weight: 164.16
Molecular Formula: $C_9H_8O_3$

Ingredient Name: Hydrogen Peroxide
CAS: 7722-84-1
Molecular Weight: 34.01
Molecular Formula: H_2O_2

HAZARDS IDENTIFICATION

For research use only, not for use in humans

INHALATION: May cause irritation. Additional effects may include coughing, chest pain and difficulty breathing.

SKIN CONTACT: May cause irritation.

EYE CONTACT: May cause irritation. Additional effects may include eye damage.

INGESTION: May cause gastrointestinal irritation. Additional effects may include digestive disorders and weakness.

ACCIDENTAL RELEASE MEASURES

In the event of a fire, evacuate area. Wear self-contained breathing apparatus and protective clothing. Use any suitable media for extinguishing the material supporting the fire (water spray, carbon dioxide, dry chemical or foam).

In the event of an occupational spill, evacuate area of unnecessary personnel, carefully sweep up material and place in a suitable container for reclamation or disposal. Ventilate area and wash spill site after pickup is completed. Observe all federal, state and local regulations.

FIRST-AID MEASURES

INHALATION: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Contact a physician.

EYES: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Contact a physician.

SKIN: In case of contact, flush skin with plenty of water. Remove contaminated clothing and shoes. Contact a physician if irritation develops and persists.

INGESTION: If swallowed, do not induce vomiting unless directed to do so by medical personnel. Give large quantities of water. Never give anything by mouth to an unconscious person. Contact a physician.

FIRE FIGHTING MEASURES

FLAMIBILITY: Nonflammable

EXTINGUISHING MEDIA: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

SPECIFIC HAZARD(S): Emits toxic fumes under fire conditions.

HANDLING AND STORAGE

HANDLING: Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.

STORAGE: Observe all federal, state and local regulations when storing this substance. Store at 4°C; keep container tightly closed when not in use.

EXPOSURE CONTROLS/PERSONAL PROTECTION

When using Luminol Reagent, the use of personal protective equipment is recommended.

PERSONAL PROTECTIVE EQUIPMENT AS FOLLOWS:

BREATHING EQUIPMENT: NIOSH/MSHA-approved respirator

PROTECTION OF HANDS: chemical-resistant rubber gloves

EYE PROTECTION: chemical safety goggles

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PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE: Liquid.

SOLUBILITY: Soluble in water.

STABILITY AND REACTIVITY

STABILITY: Stable under ordinary conditions of use and storage.

HAZADOUS POLYMERIZATION: Will not occur.

ECOLOGICAL INFORMATION

Data not yet available.

TOXICOLOGICAL INFORMATION

CARCINOGENICITY:
CAS#67-68-5: Reports of Carcinogenicity:NTP:NO IARC:NO OSHA: NO
CAS# 521-31-3: Not listed by ACGIH, IARC, NIOSH, NTP, or OSHA.
CAS# 501-98-4: Not listed by ACGIH, IARC, NIOSH, NTP, or OSHA.
CAS#7722-84-1: Reports of Carcinogenicity:NTP:NO IARC:NO OSHA: NO

DISPOSAL CONSIDERATIONS

Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

TRANSPORT INFORMATION

DOT
PROPER SHIPPING NAME: None
NON-HAZARDOUS FOR TRANSPORT: This substance is considered to be non-hazardous for transport.

IATA
Non-Hazardous for Air Transport

REGULATORY INFORMATION

United States Regulatory Information
SARA LISTED: No

Canada Regulatory Information
WHMIS Classification: The MSDS contains all the information required by the CPR.

DSL: No

NDSL: No

OTHER INFORMATION

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. Santa Cruz Biotechnology shall not be held liable for any damage resulting from handling or from contact with the product.