

SPHK2, Active

Full-length human protein expressed in Sf9 cells

Catalog # S18-102G

Lot # C2091-4

Product Description

Recombinant full-length human SPHK2 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_020126](#).

Gene Aliases

(None)

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

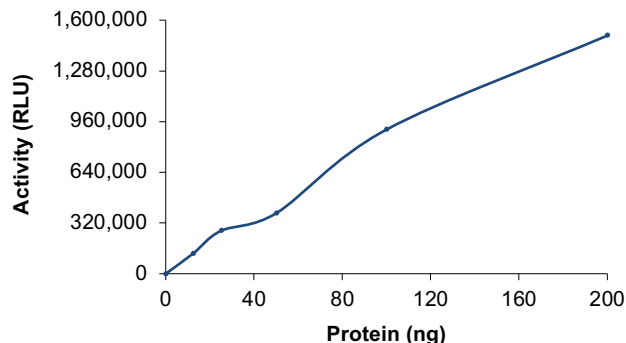
Scientific Background

SPHK2 is a member of the SPHK family and catalyzes the phosphorylation of the lipid sphingosine, creating the bioactive lipid sphingosine-1-phosphate (S1P). Sphingolipid metabolism regulates proper uterine decidualization and blood vessel stability (1). SPHK2 is involved in the regulation of chemosensitivity by controlling ceramide formation and the downstream Akt pathway in human colon cancer cells. SPHK2 plays an important role in migration of MDA-MB-453 cells toward the ligand EGF. The N-terminal-extended form of SPHK2 has a role in serum-dependent regulation of cell proliferation and apoptosis (2).

References

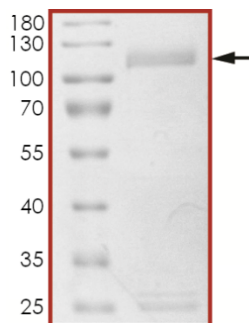
- Mizugishi, K. et al: Maternal disturbance in activated sphingolipid metabolism causes pregnancy loss in mice. J. Clin. Invest. 1172993-3006, 2007.
- Hait, N. C. et al: Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. Science 3251254-1257, 2009. NoteErratumScience 326366 only, 2009.

Specific Activity



The specific activity of SPHK2 was determined to be **168 nmol /min/mg** as per activity assay protocol.

Purity



The purity of SPHK2 was determined to be **>90%** by densitometry, approx. MW **~110kDa**.

SPHK2, Active

Full-length human protein expressed in Sf9 cells

Catalog #	S18-102G
Specific Activity	168 nmol/min/mg
Lot #	C2091-4
Purity	>90%
Concentration	0.1 µg/µl
Stability	1 yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: S18-102G)

Active SPHK2 (0.1µg/µl) diluted with Kinase Dilution Buffer IX (Catalog #: K29-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active SPHK2 for optimal results).

Kinase Dilution Buffer IX (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add freshly 50µM DTT to the aliquot prior to use.

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM MgCl₂ and 0.5 mg/ml BSA.

ADP-Glo™ Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

250 µM ATP Assay Solution

Prepare the ATP assay solution by diluting ATP solution, 10 mM (Promega, Cat # V9101) to 250 µM in Kinase Dilution Buffer IX (Catalog #: K29-09).

Substrate

Sphingosine substrate solution contains 500µM of sphingosine in Kinase Dilution Buffer IX (Catalog #: K29-09).

Assay Protocol

The SPHK2 assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the SPHK2 reaction. The ADP-Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

- Step 1.** Thaw the Active SPHK2, Kinase Assay Buffer, Substrate and Kinase Dilution Buffer on ice.
- Step 2.** In a pre-cooled 96-well opaque plate, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1.** 10µl of diluted Active SPHK2 (Catalog #S18-102G)
 - Component 2.** 5µl of 500µM Sphingosine substrate solution
 - Component 3.** 5µl of Kinase Dilution Buffer IX
- Step 3.** Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of Kinase Dilution Buffer IX.
- Step 4.** Initiate the reaction by the addition of 5µl of 250 µM ATP Assay Solution thereby bringing the final volume up to 25µl. Shake the reaction mixture in the 96-well opaque plate for 2 minutes and continue the incubation at 30°C for 15 minutes.
- Step 5.** After the 15 minute incubation period, terminate the reaction and deplete the remaining ATP by adding 25µl of ADP-Glo™ Reagent. Shake the 96-well plate and then incubate the reaction mixture for another 40 minutes at ambient temperature.
- Step 6.** Then add 50µl of the Kinase Detection Reagent to the 96-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.
- Step 7.** Read the 96-well reaction plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).
- Step 8.** Determine the corrected activity (RLU) by removing the blank control value (see Step 3) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: SPHK2, Active

Catalog # S18-102G

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: SignalChem Pharmaceuticals Inc.
Street Address: 110-13120 Vanier Place
City, Prov. Postal Code: Richmond, BC, V6V 2J2
Fax: 604-232-4601
EMERGENCY PHONE: 604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

Article 3 - Composition/Information on Ingredients

Chemical Characterization: Mixtures.

Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	25%
NaCl	Sodium chloride	7647-14-5	0.875%
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 - Amino - 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	1185-53-1	0.788%
Glutathione	Glutathione	70-18-8	0.307%
Protein		No data available	0.01%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
EDTA	Ethylenediaminetetraacetic acid	6381-92-6	0.0037%
PMSF; Phenylmethanesulfonyl fluoride	α -Toluenesulphonyl fluoride	329-98-6	0.002%

Article 4 - First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m ³ for mist	British Columbia, Canada
TWA	3 mg/m ³ for respirable mist	British Columbia, Canada
TWA	10 mg/m ³	Alberta, Canada
TWAEV	10 mg/m ³	Ontario, Canada
TWAEV	10 mg/m ³	Quebec, Canada
TWA	10 mg/m ³	USA

- **Appropriate engineering controls:**
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.
Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.
Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	Danger of explosion: Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	Explosion limits: Lower: 0.9 Vol %; Upper: 0.0 Vol %.
pH: Not available.	Decomposition temperature: Not available.
Melting point/freezing point: Not determined.	Vapor pressure at 20 °C: 0.1 hPa
Boiling point/Boiling range: 100 °C.	Density: Not determined.
Flash point: > 100 °C.	Relative density: Not determined.
Flammability (solid, gaseous): Not determined.	Vapor density: Not determined.
Ignition temperature: 400 °C.	Evaporation rate: Not determined.
Auto-igniting: Product is not self-igniting.	Solubility in / Miscibility with Water: Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may form under fire conditions; no known decomposition information for other decomposition products.

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Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion: May be harmful if swallowed.
Skin: May be harmful if absorbed through skin. May cause skin irritation.
Eyes: May cause eye irritation.
- **Signs and Symptoms of Exposure:**
Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 - Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - 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. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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