Recombinant Mouse Sphingosine Kinase 1/SPHK1

Catalog Number: 6086-SK

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived Glu2-Pro382, with an N-terminal Met and 6-His tag Accession # Q8CI15
N-terminal Sequence Analysis	Inconclusive result, Met predicted. Protein identity confirmed by MS analysis of tryptic fragments.
Structure / Form	Monomer
Predicted Molecular Mass	43 kDa

SPECIFICATIONS

SDS-PAGE	42 kDa, reducing conditions
Activity	Measured by its ability to phosphorylate Omega-(7-nitro-2-1,3-benzoxadiazol-4-yl)-D-erythro-sphingosine (NBD-sphingosine). The specific activity is >2,500 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>70%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, 250 mM NaCl , 2 mM ATP, 0.2% (v/v) Triton® X-100, 30 mM $MgCl_2$, pH 7.5
- Substrate Buffer: 4 mg/mL BSA in deionized water
- Aqueous Extraction Buffer: 1.0 M Potassium Phosphate, pH 8.5
- Organic Extraction Buffer: chloroform:methanol (2:1, v/v)
- Recombinant Mouse Sphingosine Kinase 1/SPHK1 (rmSPHK1) (Catalog # 6086-SK)
- Fluorogenic Substrate: NBD-Sphingosine (NBD-Sph) (Avanti Polar Lipids, Catalog # 810205P), 0.25 mg/mL (523 µM) stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute Substrate to 30 µM in Substrate Buffer.
2. Dilute rmSPHK1 to 0.1 µg/mL in Assay Buffer.
3. Mix 50 µL of 30 µM Substrate with 50 µL of the diluted rmSPHK1 in a microcentrifuge tube in triplicate. Include a blank consisting of 50 µL of 30 µM Substrate with 50 µL of Assay Buffer in triplicate.
4. Incubate 30 minutes at room temperature.
5. After incubation, add 100 µL of Aqueous Extraction Buffer to each reaction. Mix briefly and then add 500 µL of the Organic Extraction Buffer to each reaction.
6. Vortex at high speed for 30 seconds.
7. Centrifuge tubes in a microcentrifuge at top speed for 2 minutes to separate the phases.
8. Remove 200 µL of the aqueous (upper) phase from each tube and place into the well of a black microplate.
9. Read the plate in endpoint mode at excitation and emission wavelengths of 481 nm and 542 nm, respectively.
10. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Fluorescence* (RFU) x Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard NBD-Sphingosine-1-Phosphate (Avanti Polar Lipids, Catalog # 810207X).

Final Assay Conditions

Per Reaction:

- rmSPHK1: 0.005 µg
- Substrate: 15 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

Sphingosine kinases are cytosolic or membrane-associated enzymes that catalyze the phosphorylation of sphingosine to sphingosine-1-phosphate (S1P). Two types of sphingosine kinases, SPHK1 and SPHK2, are known to be expressed in human cells. The two enzymes share considerable amino acid sequence similarity, but differ in their N-terminal and central regions (1). The two proteins also differ in tissue distribution and some kinetic properties (1). S1P is a lipid messenger that regulates diverse physiological processes including cell proliferation, migration, apoptosis, inflammation, calcium homeostasis and cytoskeletal structure (2, 3). The level of S1P is tightly controlled by SPHKs and S1P degrading enzymes. SPHK1 and its activation can be stimulated by several growth factors such as tumor necrosis factor-α, epidermal growth factor and transforming growth factor-β (3, 4). Expression of SPHK1 has been found to increase in many human solid tumors and overexpression of SPHK1 is associated with tumor angiogenesis (5). Such studies have implicated SPHK1 as a new target for cancer treatment.

References:

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3. Alemany, R. *et al.* (2007) Naunyn-Schmiedegerg's Arch. Pharmacol. **374**:413.
4. Pederson, L. *et al.* (2008) Proc. Natl. Acad. Scis USA. **105**:20764.
5. Shida, D. *et al.* (2008) Curr. Drug Targets. **9**:662.

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