

Recombinant Human Sphingosine Kinase 1 (Full Length) with N-Terminal HIS tag Active Enzyme

BACKGROUND

Sphingosine Kinase 1 (Sphk1) catalyzes the phosphorylation of sphingosine to sphingosine 1-phosphate (S1P), an important signaling molecule with intra- and extracellular functions. Inside the cell S1P acts as a signaling molecule like other sphingolipid metabolites like ceramide and sphingosine. S1P has been implicated in regulating cell differentiation, calcium mobilization from intracellular stores, and apoptosis. The cell surface receptors for S1P are the EDG family of G protein-coupled receptors (S1P Receptors). These receptors couple to multiple G proteins (e.g. S1P₁ couples to Gi whereas S1P₂ and S1P₃ couple to Gq, G13 in addition to Gi) and regulate a extremely wide range of cellular events including cell motility, survival, apoptosis, migration and cell-cell interaction. Important roles for S1P have also been reported in regulation of cardiogenesis, vascular maturation, oocyte survival, immune cell trafficking, cells of the neuronal system and bone cells. S1P levels are regulated by the activity of Sphk (Sphk1 and Sphk2).

ORDERING INFORMATION

CATALOG NUMBER

X1708E

SIZE

10 µg

STORAGE CUSTOMER

Enzyme should be stored at -20°C.
Enzyme should be kept on ice when dispensing

STABILITY

Products are stable for one year from purchase when stored properly

SHIP CONDITIONS

Ship on gel ice, store at -20°C immediately upon arrival

FORMULATION

Provided in 25 mM Tris-HCl, 100 mM NaCl, pH 8.0, 0.05% Tween, 3 mM DTT and 50% glycerol

CONCENTRATION

Lot specific, see vial

SOURCE

Baculovirus infected Sf9 cells

ACTIVITY

Activity lot specific. See vial for specific activity of each lot. Sphingosine kinase activity was determined using sphingosine and [γ -³²P]ATP as substrates. A unit of sphingosine kinase activity is defined as the amount of enzyme required to produce 1 nmole of S1P/min.

PURITY

>80% by SDS-PAGE.

REFERENCES

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ASSAY METHODS

MATERIALS

1. Assay Buffer: 20 mM Tris (pH 7.4), 20% glycerol, 1 mM mercaptoethanol, 1 mM EDTA, 1 mM sodium orthovanadate, 40 mM b-glycerophosphate, 15 mM NaF, 10 $\mu\text{g/ml}$ leupeptin, 10 $\mu\text{g/ml}$ aprotinin, 10 $\mu\text{g/ml}$ soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride and 0.5 mM 4-deoxypyridoxine
2. [^{32}P]-ATP (10 mCi, 20 mM) containing 200 mM MgCl_2 .
3. 1N HCl
4. Chloroform/methanol/HCl (100:200:1, v/v)
5. 2M KCl
6. 1-butanol/ethanol/acetic acid/water (80:20:10:20, v/v)
7. Silica gel G60 (for TLC autoradiography)

PROCEDURE

1. Mix samples (up to 40 μg) with 10 μl of 1 mM sphingosine (dissolved in 5% Triton X-100).
2. Mix sample solution with Assay Buffer to a total volume of 190 μl .
3. Start reaction by addition of 10 μl of [^{32}P]-ATP/ MgCl_2 .
4. Incubate for 5 to 15 minutes at 37°C.
5. Terminate reaction by addition of 20 μl of 1N HCl followed by 0.8 ml of chloroform/methanol/HCl solution and vortex vigorously.
6. Add 240 μl of chloroform and 240 μl of 2M HCl.
7. Separate phases by centrifugation.
8. Resolve organic phase by TLC with 1-butanol/ethanol/acetic acid/water solution and visualize with autoradiography and counted.

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