Sphingosine Kinase 1 (Full Length) with N-Terminal HIS tag. Sf9 cells 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. $S1P_1$ couples to Gi whereas $S1P_2$ and $S1P_3$ 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

Customer Storage
Product should be stored at -80°C. Aliquot to avoid freeze/thaw cycles

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

Ship Conditions
Ship on dry ice, freeze upon arrival

Stability
Products are stable for one year from purchase when stored properly

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 [g-^{32}P]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.

Applications

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

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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 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride and 0.5 mM 4-deoxypyridoxine
2. [32P]-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 [32P-ATP/$MgCl_2$.
4. Incubate for 5 to 15 minutes at 37ºC.
5. Terminate reaction by addition of 20 μl of 1N HCl followed by 0.8 ml o 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.

References

1. Kihara A, Ikeda M, Kariya Y, Lee EY, Lee YM, Igarashi Y, Sphingosine-1-phosphate lyase is involved in the differentiation of F9 embryonal carcinoma cells to primitive endoderm. J. Biol. Chem. 278 (2003) 14578-14585

2. Choi OH, Kim JH, Kinet JP, Calcium mobilization via sphingosine kinase in signalling by the FceRI antigen receptor. Nature 380 (1996) 634-636

3. Olivera A, Kohama T, Edsall L, Nava V, Cuvillier O, Poulton S, Spiegel S, Sphingosine kinase expression increases intracellular sphingosine-1-phosphate and promotes cell growth and survival. J. Cell Biol. 147 (1999) 545-558

4. Chun J, Goetzl EJ, Hla T, Igarashi Y, Lynch KR, Moolenaar W, Pyne S, Tigyi G, International union of pharacology. XXXIV. Lysophospholipid receptor nomenclature. Pharmacol. Rev. 54 (2002) 265-269

5. Hla T, Signaling and biological actions of sphingosine 1-phosphate. Pharmacol. Res. 47 (2003) 401-407

6. Gregorius K, Mouritsen S, Elsner HI, Hydrocoating: a new method for coupling biomolecules to solid phases. J. Immunol. Methods, 181 (1995) 65-73

7. Zhang JH, Chung TDY, Oldenburg KR, A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J. Biomol. Screening, 4 (1999) 67-73

8. Olivera, A., Kohama, T., Tu, Z., Milstien, S., and Spiegel, S. (1998) J. Biol. Chem. 273, 12576–12583

9. Zhang, H., Desai, N. N., Olivera, A., Seki, T., Brooker, G., and Spiegel, (1991) J. Cell Biol. 114, 155–167

10. Olivera, A., Rosenthal, J., and Spiegel, S. (1996) J. Cell. Biochem. 60, 529–537

11. Zhang, H., Desai, N. N., Olivera, A., Seki, T., Brooker, G., and Spiegel, S. (1991) J. Cell Biol. 114, 155–167

12. Ohotski, J., et al. Expression of sphingosine 1-phosphate receptor 4 and sphingosine kinase 1 is associated with outcome in oestrogen receptor-negative breast cancer. Br. J. Can. (2012), 106, 1453-1459

Product Specific References

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