Sphingosine Kinase 2, Long Form N Terminal HIS Tag. Sf9 cells Active Enzyme

Sphk2-L, N-terminal-extended Sphingosine Kinase 2

Background

The long form of sphingosine kinase 2 (Sphk2-L) is a species-specific isoform of the Sphk2 enzyme expressed in human but not in mouse. It is an N-terminal extended form of Sphk2, extended by 36 amino acids. It functions as expected, catalyzing the formation of sphingosine 1-phosphate from sphingosine, however it appears to have a decreased ability to inhibit DNA synthesis as compared to Sphk2-S (short form of Sphk2). Under normal cellular conditions, Sphk2-L does not appear to inhibit DNA synthesis, but under serum deprivation, SphK2-L translocates to the nucleus and accumlates. This accumlation may be involved in the cessation of cell proliferation or apoptosis depending on the cell type.

Ordering Information

Catalog Number
X1840E

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, 75 mM NaCl, pH 8.0, 0.05% Tween, 5 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 for details

Source
Baculovirus infected Sf9 cells

Activity

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling. Full-length human sphingosine kinase 2, long form protein, with N-terminal His tag. 20 units/µg. Actvity determined using D-erythro-sphinogsine and ATP as substrates. One unit of activity is defined as the amount of enzyme required to produce 1 pmol of S1P/minute. Specific Activity: 20 U/ug. Sphingosine kinase activity was determined using D-erythrosphingosine
and ATP as substrates. A unit of sphingosine kinase activity is defined as

Purity

>80% by SDS-PAGE

Applications

Study of enzyme kinetics, screening inhibitors, and selectivity profiling.

Exalpha Biologicals, Inc. 2 Shaker Road, Bldg. B101 Shirley, MA 01464
Tel: 978.425.1370 www.exalpha.com info@exalpha.com

Assay Methods

Materials

1. Assay Buffer: 50 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 3 μM Na-orthovanadate, 0.5 mM ATP, 4 μM D-erythro-sphingosine and 0.75 μg/ml sphingosine kinase 2, long form
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. Okada, T., et al. 'Involvement of N-terminal-extended form of sphingosine kinase 2 in serum-dependent regulation of cell proliferation and apoptosis.' J. Biol. Chem. 2005, 280, 36318-36325

Product Specific References