



**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 accumulates. This accumulation may be involved in the cessation of cell proliferation or apoptosis depending on the cell type.

**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/ $\mu$ g. Activity determined using D-erythro-sphingosine and ATP as substrates. One unit of activity is defined as the amount of enzyme required to produce 1 pmol of S1P/minute.

**PURITY**

>80% by SDS-PAGE

**APPLICATIONS**

**ORDERING INFORMATION**

**CATALOG NUMBER**

X1840E

**SIZE**

10  $\mu$ g

**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**

Stable for > 6 months when stored as recommended.

**CONCENTRATION**

Lot specific, see vial for details

**SOURCE**

Baclovirus infected Sf9 cells

## **ASSAY METHODS**

### **MATERIALS**

1. Assay Buffer: 50 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM MgCl<sub>2</sub>, 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. [<sup>32</sup>P]-ATP (10 mCi, 20 mM) containing 200 mM MgCl<sub>2</sub>.
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 [<sup>32</sup>P]-ATP/MgCl<sub>2</sub>.
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.

### **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**

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