# Sphingosine Kinase 2 (N Terminal HIS Tag). Sf9 cells Active Enzyme

EC 2.7.1, SK 2, SPK 2, SPHK2, Gene name: SPHK2 or SPHK or SP

## Background

Sphingosine Kinase 2 (Sphk2) 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 raange 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**
X1709E

**Size**
10 µg

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

**Formulation**
Provided in 50mM Tris (pH 8) solution containing 138 mM NaCl, 10% 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 D-erythro-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

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 $\mu$g/ml leupeptin, 10 $\mu$g/ml aprotinin, 10 $\mu$g/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 $\mu$g) with 10 $\mu$l of 1 mM sphingosine (dissolved in 5% Triton X-100).
2. Mix sample solution with Assay Buffer to a total volume of 190 $\mu$l.
3. Start reaction by addition of 10 $\mu$l of [32P-ATP/$MgCl_2$.
4. Incubate for 5 to 15 minutes at 37ºC.
5. Terminate reaction by addition of 20 $\mu$l of 1N HCl followed by 0.8 ml o chloroform/methanol/HCl solution and vortex vigorously.
6. Add 240 $\mu$l of chloroform and 240 $\mu$l 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


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### Product Specific References