

Recombinant Sphingosine Kinase 2, Long Form 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.

ORDERING INFORMATION

CATALOG NUMBER
X1840E

SIZE
10 μ g

STORAGE CUSTOMER
Product should be stored at -70°C.
Aliquot to avoid freeze/thaw cycles

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

SHIP CONDITIONS
Ship on dry ice, freeze upon arrival

FORMULATION
Provided in 25 mM Tris-HCl, 75 mM
NaCl, pH 8.0, 0.05% Tween, 5 mM DTT
and 50% glycerol

CONCENTRATION
Lot specific, see vial for details

SOURCE
Baculovirus infected Sf9 cells

ACTIVITY

Full-length human sphingosine kinase 2, long form protein, with N-terminal His tag. 20 units/ μ 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

ASSAY METHODS

MATERIALS

1. Assay Buffer: 50 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM MgCl₂, 1 mM DTT, 3 μ M Na-orthovanadate, 0.5 mM ATP, 4 μ M D-erythro-sphingosine and 0.75 μ g/ml sphingosine kinase 2, long form
2. [³²P]-ATP (10 mCi, 20 mM) containing 200 mM MgCl₂.
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 μ g) with 10 μ l of 1 mM sphingosine (dissolved in 5% Triton X-100).
2. Mix sample solution with Assay Buffer to a total volume of 190 μ l.
3. Start reaction by addition of 10 μ l of [³²P]-ATP/MgCl₂.
4. Incubate for 5 to 15 minutes at 37°C.
5. Terminate reaction by addition of 20 μ l of 1N HCl followed by 0.8 ml of chloroform/methanol/HCl solution and vortex vigorously.
6. Add 240 μ l of chloroform and 240 μ 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.

For research use only. Not for use in human diagnostics or therapeutics.

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

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