



Sphingosine Kinase 1a (mouse recombinant, N-terminal His tag). Sf9 cells Active Enzyme

BACKGROUND

Sphingolipids have emerged as central signaling molecules regulating a variety of fundamental cell responses such as cell death and differentiation, proliferation and inflammation. Ceramide has been a main focus of research since it possesses pro-apoptotic capacity in many cell types. Sphingosine-1-phosphate (S1P), which is generated from ceramide by the actions of ceramidase and sphingosine kinase can potently induce cell proliferation via binding to and activation of the S1P family of receptors. The balance between ceramide and sphingosine-1-phosphate determines whether cells undergo apoptosis or proliferate, two cell responses that are critically involved in tumor development. The amino acid sequence for sphingosine kinase 1a differs from sphingosine kinase 1 only in the absence of a valine at position 4 of sphingosine kinase 1a.

ACTIVITY

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling. Full length mouse recombinant sphingosine Kinase 1a protein with N terminal His tag. 20U/ug specific activity A unit of activity determined as the amount of enzyme required to produce 1 pmol of sphingosine-1-phosphate from sphingosine and ATP in 10 minutes at 37°C.

PURITY

> 50% by SDS-PAGE

APPLICATIONS

ORDERING INFORMATION

CATALOG NUMBER

X1850E

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, 3 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

SOURCE

Baclovirus infected Sf9 cells

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 $\mu\text{g/ml}$ leupeptin, 10 $\mu\text{g/ml}$ aprotinin, 10 $\mu\text{g/ml}$ soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride and 0.5 mM 4-deoxypyridoxine
2. [^{32}P]-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 [^{32}P]-ATP/ MgCl_2 .
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. Delon, C., et al., J. Biol. Chem 279 (43) 44763-44774 (2004)
2. Taha, TA et al., FASEB J. 20(3):482-4 (2005)

PRODUCT SPECIFIC REFERENCES

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