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 kionase 1a differs from sphingosine kinase 1 only in the absence of a valine at position 4 of sphingosine kinase 1a.

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
Products are stable for one year from purchase when stored properly

Concentration
Lot Specific

Source
Baculovirus infected Sf9 cells

Activity

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity
profiling. Full lengtth mouse recombinant sphingosie Kinase 1a protein with N terminal His tag. 20U/ug specific activity A unit of activity determined as the amount ofenzyme required to produce 1 pmol of S1P/min. 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

> 50% by SDS-PAGE

Applications

Enzyme Kinetic studies, screening inhibitors, selectivity profiling

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

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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 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/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 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. Delon, C., etal., J. Biol. Chem 279 (43) 44763-44774 (2004)

2. Taha, TA etal., FASEB J. 20(3):482-4 (2005)

Product Specific References