

## Recombinant Mouse Sphingosine Kinase 1a (mouse recombinant, N-terminal His tag) 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 potentially 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.

### ORDERING INFORMATION

**CATALOG NUMBER**  
X1850E

**SIZE**  
10  $\mu$ g

**STORAGE CUSTOMER**  
Product should be stored at  $-70^{\circ}\text{C}$ .  
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, 3 mM DTT and 50% glycerol

**CONCENTRATION**  
Lot Specific

**SOURCE**  
Baculovirus infected Sf9 cells

### ACTIVITY

Full length mouse recombinant sphingosine Kinase 1a protein with N terminal His tag. 20U/ $\mu$ g specific activity A unit of activity determined as the amount of enzyme required to produce 1 pmol of S1P/min

### PURITY

#### **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. [ $^{32}\text{P}$ ]-ATP (10 mCi, 20 mM) containing 200 mM  $\text{MgCl}_2$ .
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 [ $^{32}\text{P}$ -ATP/ $\text{MgCl}_2$ .
4. Incubate for 5 to 15 minutes at  $37^{\circ}\text{C}$ .
5. Terminate reaction by addition of 20  $\mu$ l of 1N HCl followed by 0.8 ml of 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.

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

## 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)

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