

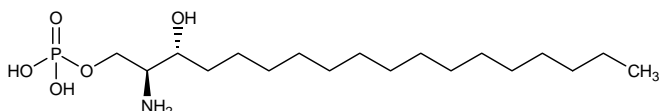
## Product Information

### D-erythro-Dihydrosphingosine 1- phosphate

Product Number **D 3439**

Storage Temperature  $-20\text{ }^{\circ}\text{C}$

Synonyms: SPP, dihydro S1P



#### Product Description

Molecular Formula:  $\text{C}_{18}\text{H}_{40}\text{NO}_5\text{P}$

Formula Weight: 381.5

Appearance: white solid

Purity: 98%

Sphingolipid metabolites, ceramide, sphingosine, and sphingosine-1-phosphate (S1P) are a class of lipid second messengers that regulate calcium mobilization, activation of phospholipase D, tyrosine phosphorylation on p125 (FAK), cell growth and survival. Various stimuli, including PDGF, activation of protein kinase C and cross-linking of the Fc $\epsilon$ RI receptor by antigens, increase cellular levels of S1P by activation of sphingosine kinase.<sup>1,2</sup> EDG-1, the receptor for S1P, binds S1P with high affinity (dissociation constant of 8.1 nM) and high specificity. In addition to signaling via EDG-1/S1P, S1P signals intracellularly to regulate cellular proliferation, cytotoxicity and suppression of apoptosis.<sup>2,3</sup>

Dihydrosphingosine-1-phosphate (dihydro-S1P) is a synthetic, structurally related, saturated analog of S1P. *In vitro* experiments with human embryonic kidney 293 fibroblasts stably expressing FLAG epitope-tagged Edg-1 (HEK293edg-1) showed dihydro-S1P blocked binding of [ $^{32}\text{P}$ ]S1P to EDG-1/S1P in a dose-dependent manner as potently as did unlabeled S1P ( $K_i = 15\text{ nM}$ ). However, dihydro-S1P had no effect on such events as mitogenesis or prevention of apoptosis and cytotoxicity, which are the result of intracellular signal transduction by S1P. Therefore, dihydro-S1P may be used as a negative control for intracellular studies of S1P signaling.<sup>3</sup>

Similar to S1P, dihydro-S1P enhances chemotaxis in human umbilical vein endothelial cells (HUVEC) which express the S1P receptor EDG-1.<sup>4</sup> Dihydro-S1P is also a ligand for other S1P receptors, including EDG-3.<sup>5</sup>

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#### Preparation Instructions

The following instructions are for preparing a solution of dihydro-S1P suitable for delivery to cells.

1. Dissolve dihydro-S1P in 60% methanol/30% tetrahydrofuran/10% water to a final concentration of 0.5 mg/ml.  
**Note:** This may require boiling ( $75\text{ }^{\circ}\text{C}$ ), with occasional replacement of evaporated methanol. Crushing of solid and stirring during heating is recommended.
2. Aliquot desired amounts of stock solution to tubes.
3. Evaporate the solvent with a stream of nitrogen, swirling to deposit a thin film of dihydro-S1P on the inside of the tube.
4. To prepare a working solution, dissolve the film prepared in step 3 using a 4 mg/ml BSA solution (fatty acid free Bovine Serum Albumin in water;  $37\text{ }^{\circ}\text{C}$ , 30 min. with repeated vortexing). A working solution of 125  $\mu\text{M}$  dihydro-S1P (47.7  $\mu\text{g/ml}$ ) is typically recommended.  
**Note:** The product is not sterile.

#### Storage/Stability

The product may be stored at  $-20\text{ }^{\circ}\text{C}$  for up to twelve months. Aliquots prepared in step 3 above may be stored at  $-20\text{ }^{\circ}\text{C}$

#### References

1. Ohanian, J. and Ohanian, V., Sphingolipids in mammalian cell signaling., *Cell Mol. Life Sci.*, **58**, 2053-2068 (2001).
2. Yatomi, Y., et al., Sphingosine 1-phosphate as a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells, *Blood*, **96**, 3431-3438 (2000).
3. Van Brocklyn, J. R. et al., Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor EDG-1 and intracellular to regulate proliferation and survival. *J. Cell. Biol.*, **142**, 229-240 (1998).

4. Wang, F., et al., Sphingosine 1-phosphate stimulates cell migration through a G<sub>i</sub>-coupled cell surface receptor. Potential involvement in angiogenesis. J. Biol. Chem., **274**, 35343-35350 (1999).
5. Van Brocklyn, J. R. et al., Sphingosine 1-phosphate-induced cell rounding and neurite retraction are mediated by the G protein-coupled receptor H218. J. Biol. Chem., **274**, 4626-4632 (1999).

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