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Data Sheet

SPHK1 Assay Kit

Catalog # 78026
96 Reactions

BACKGROUND: SPHK1 is a lipid enzyme that catalyzes the phosphorylation of sphingosine to form sphingosine 1-phosphate (SPP). It has been implicated in tumor progression through cell proliferation and motility. High levels of SPHK1 have been associated with increased mortality in various forms of human cancer.

DESCRIPTION: The *SPHK1 Assay Kit* is designed to measure SPHK1 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *SPHK1 Assay Kit* comes in a convenient 96-well format, with enough purified SPHK1, Sphingosine, ATP, and kinase assay buffer for 96 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40610	SPHK1, His-tag*	>1 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
	Sphingosine (1 mM)	100 µl	-20°C	
79696	96-well plate, white	1	RT	

*Excess material has been provided for ease of retrieval from the vial.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 0.5 M)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCES:

1. Shida, D., *et al.* 2008. "Targeting SphK1 as a new strategy against cancer." *Current Drug Targets* **9(8):** 662-673.
2. Xia, J., *et al*. 2012. "miR-124 inhibits cell proliferation in gastric cancer through down-regulation of SPHK1." *The Journal of Pathology* **227(4):** 470-480.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **Sphingosine (1 mM).** Add 30 µl of 0.5 M DTT to **5x Kinase assay buffer**.

2) Prepare the master mixture (25 µl per well): N wells x (5 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 1 µl **Sphingosine (1 mM)** + 18 µl distilled water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	5 µl	5 µl	5 µl
ATP (500 µM)	1 µl	1 µl	1 µl
Sphingosine (1 mM)	1 µl	1 µl	1 µl
Water	18 µl	18 µl	18 µl
Test Inhibitor	–	5 µl	–
Inhibitor buffer (10% DMSO in water)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
SPHK1, His-tag (0.19 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer, usually 10% DMSO in water). *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor at 10 µM that is dissolved in 100% DMSO, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

4) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

5) To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

6) Thaw **SPHK1, His-tag** on ice. Upon first thaw, briefly spin tube containing material to recover full content of the tube. Calculate the amount SPHK1, His-tag required for the assay and dilute enzyme to 0.19 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted material in aliquots at -80°C. *Note: SPHK1,*

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His-tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted material.

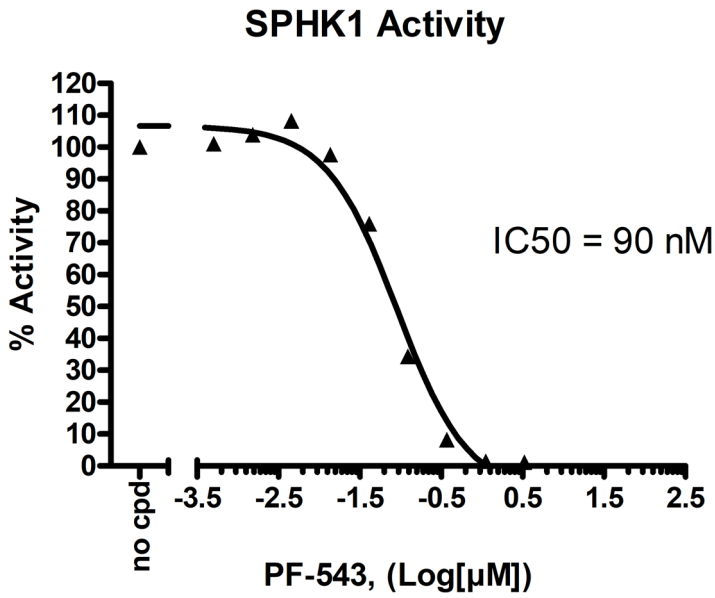
7) Initiate reaction by adding 20 µl of diluted **SPHK1, His-tag** to the wells designated “Positive Control” and "Test Inhibitor Control." Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 45-minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

10) Measure luminescence using the microplate reader. The value of the “Blank” reading should be subtracted from all other measurements.

Example of Assay Results:



Inhibition of SPHK1, His-tag by PF-543, measured using the SPHK1 assay kit (BPS Bioscience #78026). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
Sphingosine kinase 1, His-tag	40610	20 µg
5x Kinase assay buffer	79334	10 ml
ATP (500 µM)	79686	200 µl
Protein Tyrosine Kinase Substrate (poly-Glu,Tyr 4:1)	40217	1 mg
Sphingosine kinase 2, His-tag	40611	20 µg
Sphingosine kinase 1, His-tag	40610	20 µg
Sphingosine kinase 2 (long), His-tag	40612	10 µg
Mouse Sphingosine kinase 1a, His-tag	40613	10 µg
Mouse Sphingosine kinase 2, His-tag	40614	10 µg