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Data Sheet

Fluorogenic SHP1 (PTPN6) Assay Kit

Catalog #79831
Size: 96 reactions

BACKGROUND: SHP1 (also known as PTPN6) is preferentially expressed in a variety of hematopoietic cells, and is an early response gene in lymphokine-stimulated cells. The noncatalytic N-terminus of this phosphatase can interact with MAP kinases and negatively regulates ERK2 and p38 MAP-kinases activity. The PTPN6 is involved in the regulation of T cell antigen receptor (TCR) signaling, which is thought to function through dephosphorylating molecules related to the MAP kinase pathway.

DESCRIPTION: The *Fluorogenic SHP1 Assay Kit* is designed to measure SHP1 activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The SHP1 assay kit comes in a convenient 96-well format, with purified SHP1 enzyme, fluorogenic substrate, and PTP assay buffer for 100 enzyme reactions. This kit is developed to identify inhibitors of the regulatory region of SHP1.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100342	Recombinant Human SHP1 (221-523)	≥1 µg	-80°C	***Avoid freeze/ thaw cycles!***
	0.5mM PTP Substrate (DiFMUP)	50 µl	-80°C	
79716	5X PTP Assay Buffer	20 ml	-20°C	
	0.5M DTT	20 µl	-20°C	
	Black, low binding black microtiter plate	1	Room Temperature	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading exc/em=360nm/460nm

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE(S):
Gee, K.R., *et al., Anal Biochem*, 1999 Aug 15; **273(1):**41-8.
Kilgore, N.E., *et al., J. Immunol.* 2003, **170:**4891-4895.
Duchesne, C., *et al. J. Biol. Chem* 2003, **278:**14274-14283.

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

1) Prepare 1X assay buffer with 1 mM DTT from 5X assay buffer. For example, add 200 µl 5X assay buffer and 2 µl 0.5M DTT to 798 µl distilled H_2O to make 1 ml 1X assay buffer.

2) Thaw SHP1 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot SHP1 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: SHP1 enzyme is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute SHP1 in 1x assay buffer at 2 pg/µl (40 pg per reaction).

4) Add 20 µl diluted SHP1 enzyme solution to wells designated “Positive Control” and “Test Sample”. Add 20 µl 1X assay buffer to “Blank” wells.

5) Prepare the inhibitor solution.

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10 fold dilution in 1X assay buffer (at this step the compound concentration is 10-fold higher than the final concentration in 10% DMSO). If you want to an run IC50 or test lower concentrations of the compound, prepare a series of further dilutions in 1X assay buffer containing 10% DMSO (final concentration of the DMSO will be 1% in all samples).

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer.

6) Add 5 µl inhibitor solution to each well designated “Test Sample”. Add 5 µl 1X assay buffer or 10% DMSO (depending on which inhibitor solution is used) to “Blank” and “Positive Control” wells.

7) Incubate the enzyme with the compound solution at room temperature for 30 minutes.

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Component	Positive Control	Test Sample	Blank
SHP1 (2 pg/µl)	20 µl	20 µl	20 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)*	5 µl	–	5 µl
Incubate at room temperature for 30 minutes			
Substrate solution	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

8) Prepare the substrate solution: N wells × (24.5 µl 1X assay buffer (with DTT) + 0.5 µl 0.5 mM PTP Substrate).

9) Add 25 µl of the substrate solution to each well (Final concentration of the PTP substrate in a 50 µl reaction is 5 µM).

10) Incubate at room temperature for 30 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at a wavelength 360 nm and detection of emission at a wavelength 460 nm. The fluorescence intensity can also be measured kinetically. “Blank” value is subtracted from all other values.

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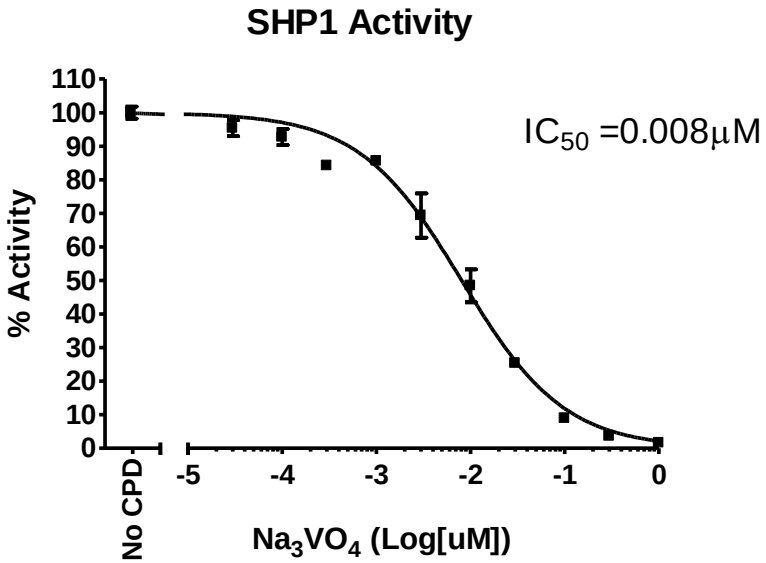
Example of Assay Results:



SHP1 enzyme activity, measured using the *Fluorogenic SHP1 Assay Kit (BPS Bioscience #79831).* Fluorescence intensity was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

Related Products

Product	Cat. #	Size
SHP-1, His-Tag	100342	20 µg
SHP-1 (PTPN6)	30021	20 µg
5X PTP Assay Buffer	79716	10 ml