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Data Sheet

Homogeneous SHP-2 Assay Kit

Catalog #79317
Size: 96 reactions

DESCRIPTION: Mammalian PTPases can be subdivided into 1 of 2 broad categories: transmembrane receptor PTPases and intracellular PTPases. SHP-2 (PTPN11) is one of the 2 closely related mammalian intracellular PTPases whose sequences encode 2 tandem SRC homology 2 (SH2) domains that are located at the amino-terminal side of a single PTPase catalytic domain. This PTP is widely expressed in most tissues and plays a regulatory role in various cell signaling events that are important for a diversity of cell functions, such as mitogenic activation, metabolic control, transcription regulation, and cell migration.

The Homogeneous SHP-2 Assay Kit is a complete assay system designed to measure SHP-2 activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent SHP-2 activity measurements. In addition, the kit includes purified SHP-2 enzyme and SHP-2 inhibitor, orthovanadate, for use as a positive and negative control. Using this kit, only one simple step on a microtiter plate is needed to analyze the SHP-2 activity level. The fluorogenic substrate, DiFMUP, is incubated with purified SHP-2 and the enzymatic activity releases DiFMU fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
30022	Recombinant Human SHP-2	1 µg	-80°C	***Avoid freeze/ thaw cycles!***
	1mM SHP-2 Substrate	50 µl	-80°C	
79626	5X SHP-2 Assay Buffer	3 ml	-20°C	
	1mM Na_3VO_4	20 µl	-80°C	
79685	*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):
Chai, J., et al., Cell, 2001 Mar 9;104(5):769-80.
Denault, JB., and Salvesen, GS., J. Biol. Chem., 2003 Sep 5;278(36):34042-50.

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

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1) Prepare 1X assay buffer from 5X assay buffer. For example, add 200µl 5X assay buffer to 800µl H_2O to make 1ml 1X assay buffer.

2) Prepare the master mixture: N wells × (19.5 µl H2O, 5 µl 5X assay buffer + 0.5 µl 1 mM SHP-2 substrate).

3) Add 25 µl of master mixture to each well (The final substrate concentration in a 50 µl reaction is 10 µM).

4) Prepare the inhibitor solution that is 10-fold higher than the final concentration.

5) Add 5 µl of the inhibitor solution to the well designed with "Test Sample". Add 5 µl of the inhibitor buffer (without inhibitor) to the wells designed with "Blank" and "Positive Control". Add 5 µl of control compound, Na_3VO_4, to the well designed with "Negative Control".

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

7) Dilute SHP-2 in 1x assay buffer at 0.2 ng/µl (4 ng per reaction).

8) Add 20 µl diluted SHP-2 solution to the wells designed with "Positive Control", "Test Sample" and Negative Control".

9) 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. "Blank" value is subtracted from all other values. You can also measure the fluorescence intensity kinetically.

Component	Positive Control	Test Sample	Negative Control	Blank
H_2O	19.5 µl	19.5 µl	19.5 µl	40 µl
5X assay buffer	5 µl	5 µl	5 µl	5 µl
Substrate	0.5 µl	0.5 µl	0.5 µl	–
Test Inhibitor	–	5 µl	–	–
Control Inhibitor	–	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	–	5 µl
SHP-2 (0.2 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	

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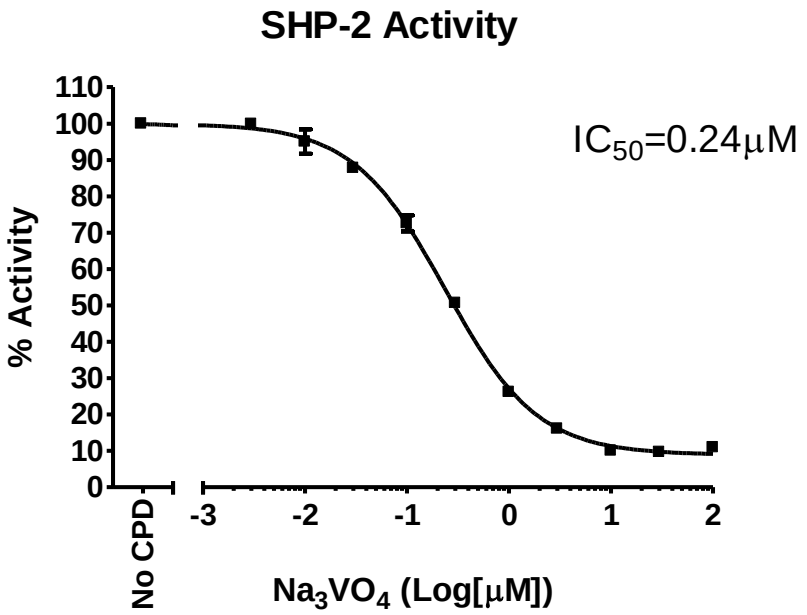
Example of Assay Results:



SHP-2 enzyme activity, measured using the *Homogeneous SHP-2 Assay Kit*, BPS Bioscience #79317. 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*

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