



# SZABO SCANDIC

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- Gefahrgutzuschlag
- Expressversand

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**Data Sheet**  
***SHP-2 (catalytic domain) Homogeneous 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 SHP-2 (catalytic domain) Homogeneous 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	<b><i>Avoid freeze/ thaw cycles!</i></b>
79769	1mM SHP-2 Substrate	50 µl	-80°C	
79626	5X SHP-2 Assay Buffer	3 ml	-20°C	
	1mM Na <sub>3</sub> VO <sub>4</sub>	20 µl	-80°C	
79685	<i>black, low binding black microtiter plate</i>	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.

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**ASSAY PROTOCOL:**

***All samples and controls should be tested in duplicate.***

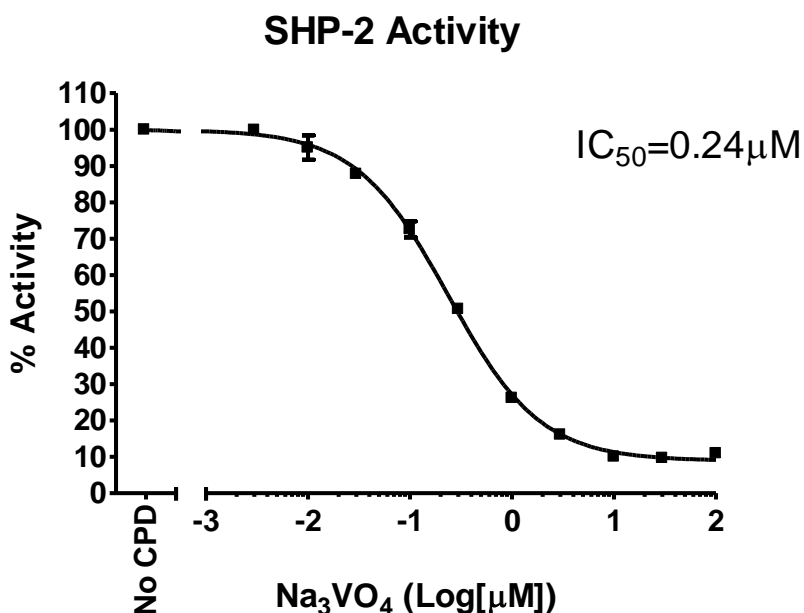
- 1) Prepare 1X assay buffer from 5X assay buffer. For example, add 200µl 5X assay buffer to 800µl H<sub>2</sub>O to make 1ml 1X assay buffer.
- 2) Prepare the master mixture: N wells x (19.5 µl H<sub>2</sub>O, 5 µl 5X assay buffer + 0.5 µl 1 mM SHP-2 substrate).
- 3) Add 25 µl of master mixture to each 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<sub>3</sub>VO<sub>4</sub>, 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.

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Component	Positive Control	Test Sample	Negative Control	Blank
H <sub>2</sub> O	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	–
<b>Total</b>	<b>50 µl</b>	<b>50 µl</b>	<b>50 µl</b>	

**Example of Assay Results:**



SHP-2 enzyme activity, measured using the *SHP-2 (catalytic domain) Homogeneous 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](mailto:info@bpsbioscience.com)*

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