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Data Sheet

Homogeneous Full Length SHP-2 Assay Kit

Catalog #79330
Size: 96 reactions

BACKGROUND: Mammalian PTPases can be subdivided into two 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 located at the N-terminal side and a single PTPase catalytic domain at C-terminus. In the inactive state, the N-terminal SH2 domain interacts with the PTP catalytic domain and blocks access of potential substrates to the active site. Upon binding to target phospho-tyrosyl residues, the N-terminal SH2 domain is released from the PTP domain, and the enzyme is activated by relieving this auto-inhibition. SHP-2 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.

DESCRIPTION: The Homogeneous full-length SHP-2 Assay Kit is a complete assay system designed to measure full length 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 full-length SHP-2 enzyme for use as a positive control. Using this kit, only two simple steps on a microtiter plate are needed to analyze the full-length SHP-2 activity level. At the first step, the SHP-2 enzyme is preincubated with SHP-2 Activating Peptide to activate the enzyme. At the second step, the fluorogenic substrate, DiFMUP, is added in the mixture and the enzymatic activity releases DiFMU fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
79018	Recombinant full length Human SHP-2	5 µg	-80°C	***Avoid freeze/ thaw cycles!***
79769	SHP-2 Substrate (1mM) (DiFMUP)	50 µl	-80°C	
79319-1	SHP-2 Activating Peptide (100 µM)	25 µl	-80°C	
79626	5x SHP-2 Assay Buffer	3 ml	-20°C	
	DTT (0.5 M)	200 µl	-20°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=360 nm/460 nm
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

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REFERENCES:

1. Chai, J., *et al., Cell*, 2001 Mar 9; **104(5):**769-80.
2. Denault, J.B., and Salvesen, GS., *J. Biol. Chem.* 2003 Sep 5; **278(36):**34042-50.
3. Fortanet, J.G., *et al., J. Med. Chem.*, 2016, **59 (17):**7773–7782

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Dilute 5x SHP-2 Assay buffer to 1x SHP-2 Assay buffer with water. Dilute only enough for the assay (undiluted 5x assay buffer is also required for the assay).
2) Dilute the 100 µM SHP-2 Activating Peptide to 5 µM using 1X assay buffer.
3) Prepare the master mixture: N wells × (8.75 µl distilled water, 5 µl 5X assay buffer, 5 µl SHP-2 activating Peptide and 0.25 µl 500 mM DTT). Add 19 µl of master mixture to each well. (The final concentration of the activation peptide is 0.5 µM, and the final concentration of DTT is 5 mM).
4) Prepare the inhibitor solution at a concentration 10-fold higher than the final desired concentration.
5) Add 5 µl of 1X assay buffer in 5% DMSO (inhibitor buffer) to the wells designed as “Test Sample.” Add 5 µl of the inhibitor buffer (without inhibitor) to the wells labeled “Blank” and “Positive Control”.
6) Thaw full length 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 the enzyme to 0.2 ng/µl using 1X assay buffer.

Component	Positive Control	Test Sample	Blank
Distilled water	8.75 µl	8.75 µl	8.75 µl
5X assay buffer	5 µl	5 µl	5 µl
SHP-2 Activating Peptide (5 µM)	5 µl	5 µl	5 µl
DTT (500 mM)	0.25 µl	0.25 µl	0.25 µl
Test Inhibitor	–	5 µl	–
1X assay buffer in 5% DMSO (inhibitor buffer)	5 µl	–	5 µl
SHP-2 enzyme (0.2 ng/µl)	1 µl	1 µl	–
1X assay buffer	–	–	1 µl
Total	**25 µl**	**25 µl**	**25 µl**

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8) Add 1 µl of the 0.2 ng/µl SHP-2 enzyme solution to the wells designed as "Test Sample" and "Positive Control". Add 1 µl of 1X assay to the wells labeled "Blank". Incubate the plate at room temperature for 1 hour.

9) During the preincubation of the enzyme and the peptide, prepare substrate solution: N wells × (19.25 µl distilled water, 5 µl 5X assay buffer, 0.25 µl 500 mM DTT and 0.5 µl 1 mM SHP-2 substrate (DiFMUP)). Add 25 µl to each well. (The final concentration of DTT is 5 mM and the substrate concentration is 10 µM)

Component	Positive Control	Test Sample	Blank
Distilled water	19.25 µl	19.25 µl	19.25 µl
5X assay buffer	5 µl	5 µl	5 µl
DTT (500 mM)	0.25 µl	0.25 µl	0.25 µl
SHP-2 Substrate (DiFMUP) (1 mM)	0.5 µl	0.5 µl	0.5 µl
Total	**25 µl**	**25 µl**	**25 µl**

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. "Blank" value is subtracted from all other values. Fluorescence intensity may also be measured kinetically.

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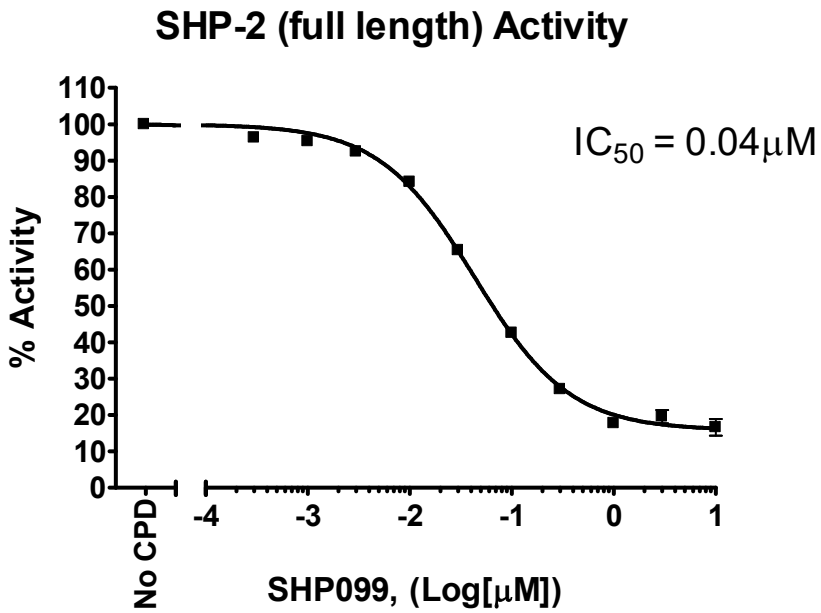
Example of Assay Results:



SHP-2 enzyme activity with an allosteric SHP-2 Inhibitor, SHP099, measured using the *Homogeneous full length SHP-2 Assay Kit*, BPS Bioscience #79330. Fluorescence intensity was measured using a Tecan Infinite M1000 fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support @bpsbioscience.com*

RELATED PRODUCTS

Product	Cat. #	Size
SHP-2 (PTPN11), GST-tag	30022	20 µg
SHP-2, His-Tag (Full Length)	79018	20 µg
SHP-1(PTPN6), GST-tag	30021	20 µg
Homogeneous SHP-2 Assay Kit	79317	96 rxns
LAR (PTPRF), GST-tag	30046	20 µg
PTPIA2(PTPRN), GST-tag	30054	20 µg
CD45(PTPRC), His-tag	30044	20 µg
PTPσ (PTPRS), GST-tag	30045	20 µg
PTPβ, GST-tag	30042	20 µg
PTPµ (PTPRM), GST-tag	30053	20 µg
RPTPγ (PTPRG), GST-tag	30047	20 µg
DEP1(PTPRJ), GST-tag	30050	20 µg
Insulin receptor (INSR), GST-tag	40241	10 µg