29-November-2019

SHP2 ELISA Protocol AB-000127

For the quantitation of SHP2 protein from human tissue, cell lines and other samples.

Please read this protocol completely prior to performing the assay.

Assay Principle

SHP2 (Src homology region 2 domain-containing phosphatase-2), a tyrosine-protein phosphatase non-receptor type 11 (PTPN11) also known as protein-tyrosine phosphatase 1D (PTP-1D),, or protein-tyrosine phosphatase 2C (PTP-2C) is an enzyme that in humans is encoded by the *PTPN11* gene. PTPN11 is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. This PTP contains two tandem Src homology-2 domains, which function as phospho-tyrosine binding domains and mediate the interaction of this PTP with its substrates. 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. Mutations in this gene are a cause of Noonan syndrome as well as acute myeloid leukemia. Patients with a subset of Noonan syndrome PTPN11 mutations also have a higher prevalence of juvenile myelomonocytic leukemias (JMML). Activating Shp2 mutations have also been detected in neuroblastoma, melanoma, acute myeloid leukemia, breast cancer, lung cancer, colorectal cancer.

In this ELISA, a series of SHP2 standards is prepared to generate a standard curve for the assay and all unknown sample concentrations should be read off this standard curve. SHP2 standards or diluted unknown samples (suggested starting sample concentration for testing 100 µg/ml) are pipetted into the provided 96-well plate which has been pre-coated with polyclonal anti-SHP2 antibodies (Capturing antibody) to capture SHP2 from testing samples. Following an incubation to allow capture of the SHP2 by the antibodies on the plate, a second biotinylated polyclonal anti- SHP2 antibody (Reporting antibody targeting a different SHP2 epitope), conjugated with biotin, is added and incubated to allow it to bind to the captured SHP2s. After 45 min. incubation, the plate is washed and a Streptavidin-HRP (Horse Radish Peroxidase) conjugate is added and incubated for 30 minutes. The Streptavidin-HRP conjugate will be captured by any biotin labeled antibody bound to the plate. Following a wash step to remove unbound conjugate, TMB substrate is added and is converted by the captured HRP to a colored product in proportion to the amount of SHP2 bound to the plate. After a short incubation to

allow color development, the reaction is stopped and the intensity of the generated color is detected in a spectrophotometer plate reader capable of measuring 450nm wavelength. A standard curve will be generated from the SHP2 standards and used to calculate the concentration of SHP2 in the unknown samples, after making suitable correction for the dilution of the sample.

Materials and Samples Preparation

PBS
Phosphate-buffered saline, pH 7.4.

PBS-T
Phosphate-buffer saline with 0.1% Tween-20.

Blocking Buffer
Provided as 2X solution.

Coating Plate
A clear plastic microtiter plate is coated with polyclonal (Capturing) anti- SHP2 IgG.

SHP2 Protein Standards
SHP2 protein stock at 4860 ng/ml is provided in 150 µL. Referring to the Assay Protocol section for details of standard preparation.

Reporting antibody
A biotin labeled polyclonal antibody is provided at 0.6 mg/mL, 150 µL/vial. Right before use, do a short centrifuge spin to collect the solution, take 100 µL and dilute into 12 ml of blocking buffer to give a 5 µg/ml working solution.

Streptavidin-HRP Conjugate
A 0.4 mL vial of Streptavidin – Horse Radish Peroxidase conjugate is provided. Immediately prior to the assay, take 0.3 mL of the HRP conjugate and dilute into 12 mL of blocking solution.

TMB Substrate
Use directly without dilution.

Stop Solution
A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution.

Plate Sealer
One.

Other Materials Required

Distilled or deionized water.

Single- and multi-channel micro-pipettes with disposable tips to accurately dispense volumes 5-250 µL.
Plastic tubes (i.e. 1.5 ml) for sample dilution
Reagent reservoirs for sample addition
Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.
Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4-parameter) fitting. Contact your plate reader manufacturer for details.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete protocol should be read and understood before attempting to use the product.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Procedural Notes

Allow diluted reagents and buffers to reach room temperature (18-25°C) prior to starting the assay. Once the assay has been started, all steps should be completed in sequence and without interruption. You do not want the plate to dry out in between steps as this can cause high backgrounds or erroneous results. Make sure that required reagents and buffers are ready when needed. Prior to adding to the plate, reagents should be mixed gently (not vortexed) by swirling.

Avoid contamination of reagents, pipette tips and wells. Use new disposable tips and reservoirs, do not return unused reagent to the stock bottles/vials and do not mix caps of stock solutions.

Incubation time can affect results. All wells should be handled in the same order for each step.

Microplate washing is important and can affect results by giving erroneous results or high backgrounds. We recommend a multichannel pipette or use wash station to add 250 µl of buffer to each well across the plate, followed by a dumping out of contents (to a sink or other receptacle) with a rapid wrist motion. The plate should then be tapped firmly on a paper towel

to shake out any remaining liquid. Avoid prolonged incubation is wash buffer when performing wash steps.

When making additions to the plate, be careful to avoid damaging the coating, for example by scratching the bottoms or the sides of the wells. One technique to avoid this is to make additions (for a right-handed person) from left to right across the plate, supporting the pipette tips on the right edge of the well with each addition and thus avoiding contact with the bottom or sides of the wells.

During the incubation times, the plate should be covered to minimize evaporation from the wells. This can be done with the adhesive covers provided or by stacking an empty plate on top.

After the last wash step and prior to adding the TMB substrate, wipe the bottom of the plate with a clean paper towel to ensure that moisture or fingerprints do not interfere with the OD reading.

Once the TMB substrate is added it will be converted by the captured HRP to a blue colored product. Generally, we find that a 15 minutes incubation is sufficient for enough color development to discern differences between the standards and the reaction should be stopped at this point. Bear in mind that, given sufficient time, even a small amount HRP is capable of converting all the TMB to product and if this happens it will be difficult to discern differences between differing concentrations of LON. Keeping OD_{450} values well below 2.0 will result in greatest accuracy as at high absorbance values very little light is reaching the detector and measurements are error prone. (Remember that at an OD of 1.0 only 10% of the light is being detected and at an OD of 2.0 only 1% of the light is reaching the detector).

Assay Protocol

1. SHP2 standard preparation: take out 8 siliconized Eppendorf centrifugation vials (1.7 ml), mark the vials as 810 ng/ml, 270 ng/ml, 90 ng/ml, 30 ng/ml, 10 ng/ml, 3.3 ng/ml , 1.1 ng/ml and 0 ng/ml respectively. Add 500 µl of blocking solution to the vial marked 810 ng/ml, add 400 µl of blocking buffer to each of the rest vials. SHP2 protein stock at 4860 ng/ml is provided in 150 µL. After a short centrifuge spin to collect the solution in the vial, take 100 µl of standard stock and add to the 500 µl of blocking buffer in the vial marked 810 ng/ml, mix by inverting the vial a few times. Take 200 µl solution from the 810 ng/ml tube and add to the vial marked 270 ng/ml for a 1:3 dilution, repeat the process to dilute down to the 1.1 ng/ml vial. Take 200 µl from the 1 ng/ml and discard. Now all the vials should have 400 µl solution.

2. Use the plate layout sheet on the back page to plan sample layout on plate and also aid in proper sample and standard identification after the assay. We recommend that

assays are carried out in duplicate or (preferably) triplicate in order to minimize spurious results.

3. Testing sample preparation: dilute your sample in 1x blocking buffer to make it at 100 µg/mL (this can be adjusted based on the SHP2 expression level), for purified SHP2, we recommend to use 500 ng/mL protein concentration. Make 400 µL for each testing sample.

4. Add both the SHP2 standards and testing samples based on the plate layout, incubate for 60 min. at room temperature.

5. During the above incubation, dilute the reporting antibody to 5 µg/ml in 12 ml of 1x blocking Buffer.

6. Wash plate by emptying contents and adding 250 µL of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.

7. Pipette 100 µL of Reporting Antibody into each well. Cover plate and incubate plate 45 min. at room temperature.

8. During the above incubation, dilute the Streptavidin-HRP conjugate to 0.1 µg/ml in 12 ml of 1x Blocking Buffer.

9. Wash plate by emptying contents and adding 250 µL of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.

10. Pipette 100 µL of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate 30 min. at room temperature.

11. Wash plate by emptying contents and adding 250 µL of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.

12. Add 100 µL of TMB substrate to each well. Monitor color development and stop reaction by adding 100 µL of Stop Solution to each well when color development within standards is sufficient. Generally 15 minutes time will be sufficient.

13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

14. Either graph the results on log graph paper or use the plate reader's built-in 4-parameter fit software capabilities to calculate SHP2 concentration for each sample.

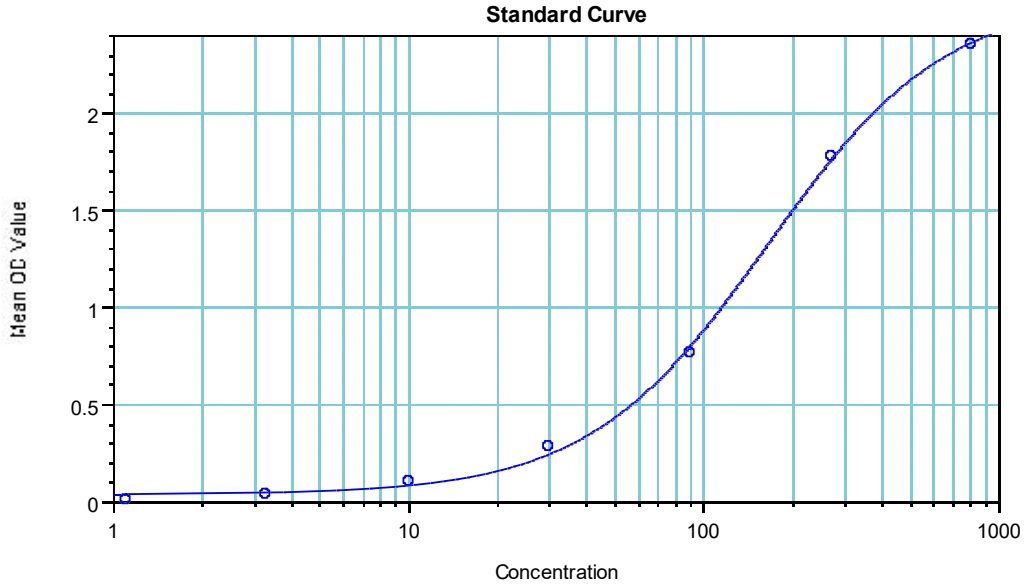
Calculation of Results

Average the triplicate OD readings for each standard, sample and background wells to give a mean OD reading. Subtract the averaged background values from the mean OD values to give a net OD value and create a standard curve using either log graph paper of 4-parameter fit software. Match OD values for the unknowns to [HCP] using the standard curve, remembering to correct for dilution.

Typical data

Sample	Mean OD	[SHP2]
1.1 ng/ml SHP2	0.009	
3.3 ng/ml SHP2	0.037	
10 ng/ml SHP2	0.100	
30 ng/ml SHP2	0.284	
90 ng/ml SHP2	0.763	
270ng/ml SHP2	1.781	
810ng/ml SHP2	2.352	
Unknown 1		
Unknown 2		
Unknown 3		

Typical Standard Curve



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
○	STD#1 (Standards: Concentration vs Mean OD Value)	0.036	1.434	163.256	2.595	0.999

Plate Template

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								