Date: 29 Nov. 2019

SHP2Bridge ELISA Kit

1 Plate Kit Catalog # AB000220

Complete kit for the systematic characterization of wild type and mutant SHP2 Higher Order Structure in human tissue, cell lines and other type of samples

Please read this insert completely prior to performing the assay.
This kit is intended for research use only. Not for use in diagnostic procedures.

Background information

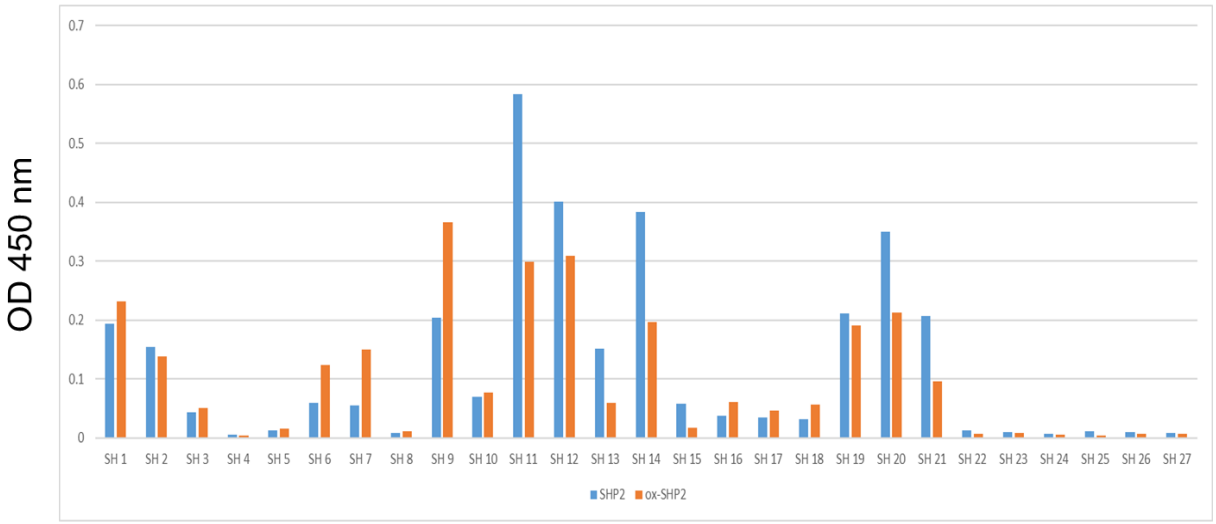
SHP2 (Src homology region 2 domain-containing phosphatase-2), a tyrosine-protein phosphatase non-receptor type 11 (PTPN11) also known as protein-tyrosine phosphatase 1D (PTP-1D),, or protein-tyrosine phosphatase 2C (PTP-2C) is an enzyme that in humans is encoded by the *PTPN11* gene. PTPN11 is a member 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.

Assay Principle

The assay is in a sandwich ELISA format where the plate is pre-coated with a panel of 27 antibodies raised against peptides derived from the full length protein sequence of SHP2, a total of 27 columns will be used, each column is pre-coated with one specific antibody covering one region of SHP2 (about 30 amino acids in length). Taken individually, each of these antibodies is strongly antigenic to the peptide sequence that was used in its production. However, when these peptides are incorporated into a full length correctly folded protein, the antigenicity of some of them is masked by the three dimensional structure (Higher Order Structure, or HOS) of the protein and only a certain number of the antibodies respond. The result is a histogram which can be likened to a 'fingerprint' for correctly folded SHP2. For a testing sample from cancer tissue, cell line or purified SHP2, if the protein is not mutated or the mutations do not impact the SHP2 HOS, the 'fingerprint' will match that of wild type SHP2. If the SHP2 mutations or a screened small molecule drug candidate resulted in the alteration of the protein HOS, , changes in the 'fingerprint' generated by the ELISA will point out differences quantitively, this information may be valuable in the characterization of the SHP2 from cancer patient samples, cancer cell lines, other biological samples or the identification of small molecules that are SHP2 HOS disruptors (allosteric regulators).

The assay is performed by making a 100 µg/ml solution of testing sample and 0.50 µg/ml of SHP2 reference material respectively and adding to the 96-well plate (for the testing of small molecule candidates that are potential HOS disruptors, only purified SHP2 will be used). Following a 1-hour incubation to allow capture of the SHP2 proteins by the panel of antibodies on the plate (at this stage, the small molecule can be added to the sample), a reporting polyclonal anti-SHP2 antibody, conjugated with biotin, is added and incubated for 45 min.(to allow it to bind to any captured SHP2 proteins, at this step, the small molecule HOS disruptor may also be included) . After this 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 HRP 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 microtiter plate reader capable of measuring 450 nm wavelength. The color development will be proportional to the captured Biosimilar or SHP2 reference protein. A typical ELISA with only the SHP2 reference protein is shown in the graph below. Your results may differ from this as your source sample will not be the same one that we used to generate this plot.

SHP2 HOS Analysis with SHP2 PCA ELISA



Antibody Number

Supplied Components:

Coated Clear 96 Well Plates
3 clear plastic microtiter plates coated with the panel of SHP2 antibodies, each antibody covering a defined SHP2 region was coated in one column. Plate 1 includes antibodies 1-10 (corresponding to columns 1-10, starting from the N-terminal of SHP2), Plate 2 includes the antibodies 11-20 (corresponding to columns 1-10), and plate 3 includes the antibodies 21-27 (corresponding to columns 1-7, this is the C-terminal part of SHP2).
Kit AB-000220 (3 plates)

2x Dilution Buffer
Buffer used for dilution of antibodies and Streptavidin-HRP conjugate. The 50 ml of concentrate should be diluted to 100 ml with 50 ml deionized or distilled water.
Kit AB-000220(50 ml)

10x PBS-T
After dilution, it is used as wash solution. The 50 ml of concentrate should be diluted to 500 ml with 450 ml deionized or distilled water.
Kit AB-000220(50 ml)

Reporting antibody
A biotin labeled polyclonal antibody against SHP2. Immediately prior to the assay, do a short spin of the Reporting antibody vial, take 200 µl and dilute into 30 ml of 1x Dilution buffer to give a working solution.
Kit AB-000220 (250 µl / tube)

Streptavidin-HRP Conjugate
A Streptavidin – Horse Radish Peroxidase conjugate in a special stabilizing solution. Immediately prior to the assay, dilute the entire 750 µl into 30 ml of 1x Dilution buffer to give a 0.1 µg/ml working stock.
Kit AB-000220(4 µg/ml, 750 µl / tube)

TMB Substrate
Use directly without dilution.
Kit AB-000220(30 ml)

Stop Solution
A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution.
Kit AB-000220(30 ml)

Plate Sealer
Kit AB-000220(three)

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 – 15 ml) for sample dilution
Reagent reservoirs for sample addition
Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert 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**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 4

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 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 with wash buffer when performing wash steps.

When making additions to the plate, be careful to avoid damaging the antibody 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 10 to 15 minute 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. 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. Use the plate layout sheet on the back page to plan sample layout on plate and also aid in proper sample and antibody identification after the assay. Each plate is laid out as shown on the plate maps on the following pages, with each unique antibody appearing in 6 positions on the plate. Rows A and H are not used in order to minimize edge effects. We recommend that assays are carried out in duplicate or (preferably) triplicate in order to minimize spurious results. For example, we have shown the plate layout for an experiment in triplicate, where the wells used for the control compound are highlighted and the three rows underneath are used for the test compound. For an experiment in duplicate, use rows B-C for the control and rows D-E and F-G for two test compounds.

2. Dilute the 10xPBS-T and 2x Dilution buffer with water to 1x-strength. Check both concentrate bottles for precipitates before proceeding and if found warm slightly in a water bath to dissolve before proceeding. The 50 ml of 10xPBS-T should be diluted to 500 ml with 450 ml water and the 50 ml of 5x Dilution Buffer should be diluted to 100 ml with 50 ml water.

3. Dilute your sample to a concentration of 100 µg/mL (this is the recommended protein concentration, it can be adjusted based on the level of SHP2 in the sample) and SHP2 standard to a concentration of 0.1 µg/ml; prepare at least 5 ml of each if samples are to be run in duplicate, 8 ml of each if run in triplicate. Pipette 100 µL of sample or SHP2 standard into each row of the plate. For replicates use multiple rows, i.e. SHP2 standard in rows 2-3, sample 1 in rows 4-5 and sample 2 in rows 6-7. Cover plates and incubate 1 hour at room temperature.

4. During the above incubation, dilute the reporting antibody by adding 100 µL to 25 ml of Dilution Buffer.

5. 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.

6. Pipette 100 µL of 5 µg/ml Reporting Antibody into each well. Cover plate and incubate plate 45 min. at room temperature.

7. During the above incubation, dilute the 4 µg/ml Streptavidin-HRP conjugate to 0.1 µg/ml by adding the entire 750 µL to 30 ml of Dilution Buffer.

8. 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.

9. Pipette 100 µL of 0.1 µg/ml Streptavidin-HRP conjugate into wells. Cover plate and incubate plate 45 min hour at room temperature.

10. 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 2 more times

11. Add 100 µL of TMB substrate to each well. Allow color development to proceed for exactly 15 minutes and then stop reaction by adding 100 µL of Stop Solution to each well. Upon addition of stop solution, developed color will change from blue to yellow.

12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm, Use wells H10-H12 as blank.

13. Export the plate reader data into Excel and calculate an average and variance for each set of replicates. If the variance is large inspect the raw data to determine the problem. With data in triplicate, one outlier may be evident, but if data is in duplicate, the higher value is generally suspect (it's easier to get a high value in error than a low value). Graph the data as a bar graph so that for each array antibody the response can be compared between your sample and SHP2 standard. Any differences between your sample and the SHP2 standard should be apparent.

Plate 1 Template (N-terminal Part of SHP2)

Control compound suggested use in wells marked

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Blank	Blank
C	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Blank	Blank
D	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Blank	Blank
E	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Blank	Blank
F	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Blank	Blank
G	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Blank	Blank
H												

Plate 2 Template (middle part of SHP2)

Control compound suggested use in wells marked

	A	B	C	D	E	F	G	H
1		Ab11	Ab11	Ab11	Ab11	Ab11	Ab11	
2		Ab12	Ab12	Ab12	Ab12	Ab12	Ab12	
3		Ab13	Ab13	Ab13	Ab13	Ab13	Ab13	
4		Ab14	Ab14	Ab14	Ab14	Ab14	Ab14	
5		Ab15	Ab15	Ab15	Ab15	Ab15	Ab15	
6		Ab16	Ab16	Ab16	Ab16	Ab16	Ab16	
7		Ab17	Ab17	Ab17	Ab17	Ab17	Ab17	
8		Ab18	Ab18	Ab18	Ab18	Ab18	Ab18	
9		Ab19	Ab19	Ab19	Ab19	Ab19	Ab19	
10		Ab20	Ab20	Ab20	Ab20	Ab20	Ab20	
11		Blank	Blank	Blank	Blank	Blank	Blank	
12		Blank	Blank	Blank	Blank	Blank	Blank	

Plate 3 Template (C-terminal part of SHP2)

Control compound suggested use in wells marked

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Ab21	Ab22	Ab23	Ab24	Ab25	Ab26	Ab27	Blank	Blank	Blank	Blank	Blank
C	Ab21	Ab22	Ab23	Ab24	Ab25	Ab26	Ab27	Blank	Blank	Blank	Blank	Blank
D	Ab21	Ab22	Ab23	Ab24	Ab25	Ab26	Ab27	Blank	Blank	Blank	Blank	Blank
E	Ab21	Ab22	Ab23	Ab24	Ab25	Ab26	Ab27	Blank	Blank	Blank	Blank	Blank
F	Ab21	Ab22	Ab23	Ab24	Ab25	Ab26	Ab27	Blank	Blank	Blank	Blank	Blank
G	Ab21	Ab22	Ab23	Ab24	Ab25	Ab26	Ab27	Blank	Blank	Blank	Blank	Blank
H												