P53Bridge ELISA Kit

1 Plate Kit Catalog # AB00219

Complete kit for the systematic characterization of wild type and mutant p53 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

The p53 ELISA kit is designed to quantitatively measure p53 from human tissues, cells or other types of sample. Tumor protein p53, also known as p53, cellular tumor antigen p53 (UniProt name), phosphoprotein p53, tumor suppressor p53, antigen NY-CO-13, or transformation-related protein 53 (TRP53), is any isoform of a protein encoded by homologous genes in various organisms, such as *TP53* (humans) and *Trp53* (mice). This homolog (originally thought to be, and often spoken of as, a single protein) is crucial in multicellular organisms, where it prevents cancer formation, and thus functions as a tumor suppressor. As such, p53 has been described as "the guardian of the genome" because of its role in conserving stability by preventing genome mutation. Hence *TP53* is classified as a tumor suppressor gene. The name p53 was given in 1979 describing the apparent molecular mass; SDS-PAGE analysis indicates that it is a 53-kilodalton (kDa) protein. However, the actual mass of the full-length p53 protein (p53α) based on the sum of masses of the amino acid residues is only 43.7 kDa. an acidic N-terminus transcription-activation domain (TAD), also known as activation domain 1 (AD1), which activates transcription factors. The N-terminus contains two complementary transcriptional activation domains, with a major one at residues 1–42 and a minor one at residues 55–75, specifically involved in the regulation of several pro-apoptotic genes. The protein can be divided into several functional domains: 1. activation domain 2 (AD2) important for apoptotic activity: residues 43-63. 2. proline rich domain important for the apoptotic activity of p53 by nuclear exportation via MAPK: residues 64-92. 3. central DNA-binding core domain (DBD). Contains one zinc atom and several arginine amino acids: residues 102-292. This region is responsible for binding the p53 co-repressor LMO3. 4. Nuclear Localization Signaling (NLS) domain, residues 316-325. homo-oligomerisation domain (OD): residues 307-355. Tetramerization is essential for the activity of p53 *in vivo*. 5. C-terminal involved in downregulation of DNA binding of the central domain: residues 356-393.

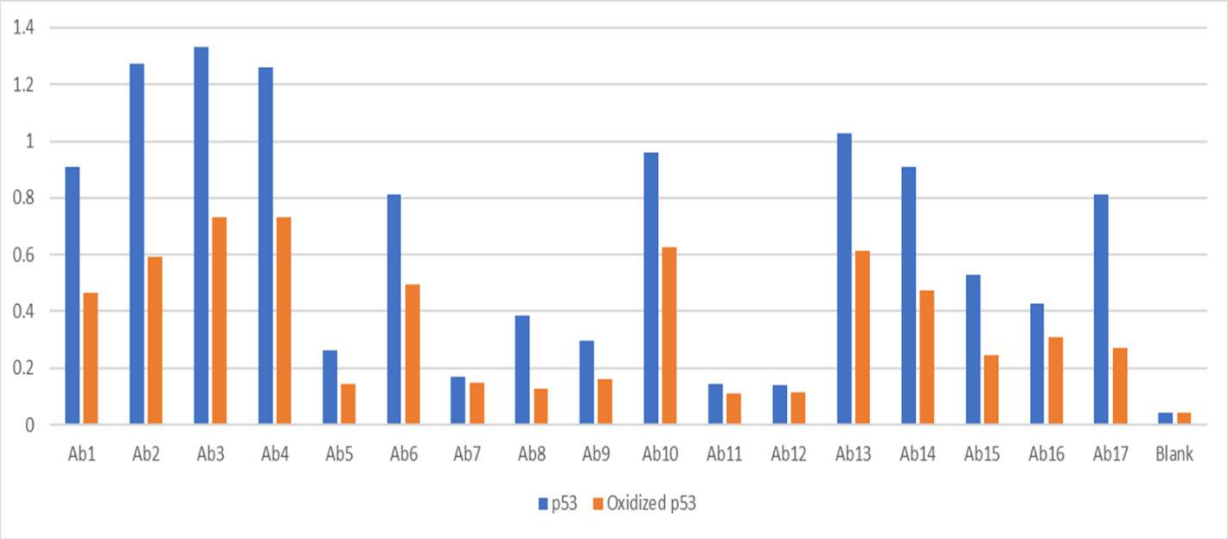
Mutations that deactivate p53 in cancer usually occur in the DBD. Most of these mutations destroy the ability of the protein to bind to its target DNA sequences, and thus prevents transcriptional activation of these genes. As such, mutations in the DBD are recessive loss-of-function mutations. Molecules of p53 with mutations in the OD dimerise with wild-type p53, and prevent them from activating transcription. Therefore, OD mutations have a dominant negative effect on the function of p53. Wild-type p53 is a labile protein, comprising folded and unstructured regions that function in a synergistic manner

Assay Principle

The assay is in a sandwich ELISA format where the plate is coated with a panel of 17 antibodies raised against peptides derived from the full length protein sequence of p53. 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 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 p53. For a testing sample from cancer tissue or cell line, if the protein is not mutated or the mutations do not impact the p53 HOS, the 'fingerprint' will match that of wild type p53. If the p53 mutations resulted in the alteration of the protein HOS, , changes in the 'fingerprint' generated by the ELISA will point out differences between the mutant and wild type p53, this information may be valuable in the characterization of the p53 from cancer patient samples, cancer cell lines or other biological samples .

The assay is performed by making a 100 µg/ml solution of testing sample and 0.10 µg/ml of p53 reference material respectively and adding to the 96-well plate. Following an 1 hour incubation to allow capture of the p53 proteins by the panel of antibodies on the plate, a reporting polyclonal anti-p53 antibody, conjugated with biotin, is added and incubated for 45 min. to allow it to bind to any captured p53 proteins. 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 p53 reference protein. A typical ELISA with only the p53 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.

p53 Conformational Array ELISA



Supplied Components:

Coated Clear 96 Well Plates
2 clear plastic microtiter plates coated with the panel of p53 antibodies. Plate 1 covers the antibodies 1-10 (corresponding to columns 1-10) and plate 2 covers the antibodies 11-17 (corresponding to columns 1-7).
Kit AB-000219 (2 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-000219(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-000219(60 ml)

Reporting antibody
A biotin labeled polyclonal antibody against p53. Immediately prior to the assay, do a short spin of the Reporting antibody vial, take 100 µl and dilute into 25 ml of 1x Dilution buffer to give a 5 µg/ml working stock.
Kit AB-000219(1.25 mg/ml, 150 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-000219(4 µg/ml, 750 µl / tube)

TMB Substrate
Use directly without dilution.
Kit AB-000219(30 ml)

Stop Solution
A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution.
Kit AB-000219(30 ml)

Plate Sealer
Kit AB-000219(two)

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 p53 in the sample) and p53 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 p53 standard into each row of the plate. For replicates use multiple rows, i.e. p53 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 P53 standard. Any differences between your sample and the P53 standard should be apparent.

Plate 1 Template (variable region)

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	Ab11	Ab12
C	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12
D	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12
E	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12
F	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12
G	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12
H												

Plate 2 Template (constant region-1)

Control compound suggested use in wells marked

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Ab13	Ab14	Ab15	Ab16	Ab17	Ab18	Ab19	Ab20	Ab21	Ab22	Ab23	Ab24
C	Ab13	Ab14	Ab15	Ab16	Ab17	Ab18	Ab19	Ab20	Ab21	Ab22	Ab23	Ab24
D	Ab13	Ab14	Ab15	Ab16	Ab17	Ab18	Ab19	Ab20	Ab21	Ab22	Ab23	Ab24
E	Ab13	Ab14	Ab15	Ab16	Ab17	Ab18	Ab19	Ab20	Ab21	Ab22	Ab23	Ab24
F	Ab13	Ab14	Ab15	Ab16	Ab17	Ab18	Ab19	Ab20	Ab21	Ab22	Ab23	Ab24
G	Ab13	Ab14	Ab15	Ab16	Ab17	Ab18	Ab19	Ab20	Ab21	Ab22	Ab23	Ab24
H												