p53 ELISA KIT

INTENDED USE

The p53 ELISA is to be used for the in-vitro quantitative determination of p53 in serum, plasma, or cell lysates. The assay will recognize both natural and recombinant p53. The kit recognizes p53 to different extend depending on the source of protein **This kit has been configured for research use only and is not to be used in diagnostic procedures.**

PRINCIPLE OF THE METHOD

The p53 Kit is a solid phase sandwich Enzyme Linked-Immuno- Sorbent Assay (ELISA). A monoclonal antibody specific for p53 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known p53 concentrations and unknowns are pipetted into these wells.
During the first incubation, the p53 antigen is added to wells. After washing, a biotinylated monoclonal antibody specific for p53 is incubated. Then the enzyme (streptavidin-peroxydase) is added. After incubation and washing to remove all unbound enzyme, a substrate solution which actes on the bound enzyme is added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of p53 present in the samples.

REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°c)	COLOUR CODE	1x96 wells Cat # 850.630.096	2x96 wells Cat # 850.630.192	RECONSTITUTION
96-wells microtiter plates		1	2	Ready-to-use
Plastic cover		2	4	
Standard : 100 U/ml	Yellow	2 vials	4 vials	Reconstitute with the volume of standard diluent indicated on the vial. (See Reagents Preparation on page 2)
Control	Silver	2 vials	4 vials	Reconstitute with the volume of standard diluent indicated on the vial. (See Reagents Preparation on page 2)
Standard Diluent : buffer	Black	1 vial	1 vial	(25 ml) 10X concentrate. Dilute in distilled Water.
Standard Diluent : human serum	Black	1 vial	2 vials	(7 ml) Ready-to-use
Biotinylated anti-p53	Red	1 vial	2 vials	(0.4 ml) Dilute in biotinylated antibody diluent
Biotinylated Antibody Diluent	Red	1 vial (7.5 ml)	1 vial (13 ml)	Ready-to-use
Streptavidin-HRP		2 vials	4 vials	(5 µl) 0.5ml of HRP-Diluent before further dilutions
HRP Diluent	Red	1 vial	1 vial	(23 ml) Ready-to-use
Washing Buffer	White	1 vial	2 vials	(10 ml) 200X concentrate. Dilute in distilled Water
Chromogen TMB :		1 vial (11 ml)	1 vial (24 ml)	Ready-to-use
H2SO4 : Stop Reagent	Black	1 vial	2 vials	(11 ml) Ready-to-use

MATERIAL REQUIRED BUT NOT PROVIDED

* Distilled water.
* Pipettes : 10 µl, 50 µl, 100 µl, 200 µl and 1000 µl.
* Vortex mixer and magnetic stirrer.

SAFETY

* For research use only.
* The human blood components included in this kit have been tested and found non reactive for HBsAg and anti-HIV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures , e.g. CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984.
  * Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water.
  * Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
  * Do not pipette by mouth.

PROCEDURAL NOTES/LAB. QUALITY CONTROL

1. When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
2. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration.
3. Cover or cap all reagents when not in use.
4. Do not mix or interchange reagents between different lots.
5. Do not use reagents beyond the expiration date of the kit.
6. Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination ; for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts.
7. Use a clean plastic container to prepare the washing solution.
8. Thoroughly mix the reagents and samples before use by agitation or swirling.
9. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
10. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
11. If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
12. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
13. Respect incubation times described in the assay procedure.
14. Dispense the TMB solution within 15 min. following the washing of the microtiter plate.

SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants- Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.
Serum–Avoid any inintentional stimulation of the cells by the procedure. Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clothing. For that, after clothing, centrifuge at approximately 1000 x g for 10 min and remove serum.
Plasma-EDTA, citrate and heparin plasma can be assayed. Spin samples at 1000 x g for 30 min to remove particulates. Harvest plasma.
Storage-If not analyzed shortly after collection, samples should be aliquoted (250-500µl) to avoid freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens.
When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present, this should be removed prior to assay by centrifugation or filtration.
Recommendation : Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before assaying

PREPARATION OF REAGENTS

Standard buffer diluent10X concentrate
Dilute 10 times with distilled water before use.

Standards
Depending on the type of samples you are assaying, the kit includes two standard diluents. Because biologicals fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure. You should reconstitute standard vials with the most appropriate standard diluent.
For serum and plasma samples use standard diluent : human serum and for cells culture supernatants use Standard diluent buffer. Standard vials have to be reconstituted with the volume of appropriate standard diluent indicated on the vial. This reconstitution gives a stock solution of 100 U/ml p53. Allow standard to stand for 5 minutes with gentle swirling prior to making dilutions. Serial dilutions of standard must be made before each assays and cannot be stored.

Controls
Freeze-dried control vials should also be reconstituted with the most appropriate diluent to your samples. For serum and plasma samples use standard diluent : human serum and for cells culture supernatants use Standard diluent buffer. Control vials have to be reconstituted with the volume of standard buffer diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution for which the p53 concentration is stated on the vial. Allow control to stand for 5 minutes with gentle swirling prior to distribute in control wells. Do not store after use.

Dilution of biotinylated anti-p53

Preparation immediately before use is recommended. Dilute the biotinylated anti-p53 with the biotinylated antibody diluent in a clean glass vial according to the number of wells to be used. See the next table for volumes to pipette. Extemporaneous preparations are recommended.

Number of Wells used	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

Dilution of Streptavidin-HRP

Add 0.5 ml of HRP diluent to a 5 µl vial of Streptavidin-HRP . DO NOT KEEP THIS DILUTION FOR FURTHER EXPERIMENTS. Dilute immediately before use. Following the number of wells to be used, further dilutions of Streptavidin-HRP should be made with HRP diluent in a clean glass vial : see hereafter the table for volumes to pipette.

Number of Wells	Streptavidin-HRP(µl)	Strep-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

Washing Buffer 200X concentrate

Dilute 200 times in distilled water.

ASSAY METHOD

a) Before use, mix all reagents thoroughly without making foam.

b) Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control samples should be assayed **in duplicate**. Remove sufficient microwell strips from the pouch.

c) Add 100 µl of appropriate standard diluent (see preparation of reagents) to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter reagents preparation. Pipet 200 µl of standard into wells A1 and A2 (see Plate Scheme below). Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of p53 standard dilutions ranging from 100 to 3.12 U/ml. Discard 100 µl from the content of the last microwells used (F1, F2).
Alternatively these dilutions can be done in separate tube and diluted standard pipetted directly into wells.

d) Add 100 µl of appropriate standard diluent to the blank wells (G1-G2)

e) Add 100 µl of sample to sample wells and 100 µl of the reconstituted control vial to the control wells (H1,H2).

f) Cover with a plate cover and incubate for 2 hours at room temperature (18°C - 25°C).

g) Remove the cover and wash the plate as follows:
   1) aspirate the liquid from each well ;
   2) dispense 0.3 ml of washing solution into each well ;
   3) aspirate again the content of each well ;
   4) Repeat steps 2) and 3) two times.

h) Preparation of biotinylated anti-p53 : (see preparation of reagents).

i) Add 50 µl of diluted biotinylated anti-p53 to all wells.

j) Cover and incubate 1 hour at room temperature.

k) Wash as described in point g)

l) Prepare HRP solution just before use: (see preparation of reagents).

m) Dispense 100 µl of HRP solution into all wells, including the blank wells. Put back the cover.

n) Incubate the microwell strips at room temperature for 30 minutes.

o) Remove plate cover and empty wells. Wash microwell strips according to point (g). Proceed immediately to the next step.

p) Pipette 100 µl of ready-to-use TMB substrate solution into all wells, including the blank wells and incubate in the dark for 15-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.

q) Incubation time of the substrate solution is usually determined by the ELISA reader performances: many ELISA readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly readable (maximum 20 minutes).

r) The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of H_2SO_4 : stop reagent into each well, including the blank wells, to completely and uniformly inactivate the enzyme. Results must be red immediately after the addition of H_2SO_4 : stop reagent, or within one hour, if the microwell strips are stored at 2-8°C in the dark.

s) Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

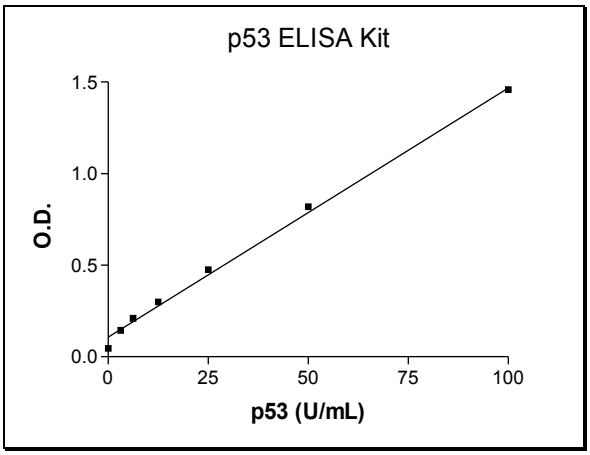
SUGGESTED PLATE SCHEME

Standard Concentrations U/mL — Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100										
B	50	50										
C	25	25										
D	12.5	12.5										
E	6.25	6.25										
F	3.12	3.12										
G	Blank	Blank										
H	CTRL	CTRL										

DATA ANALYSIS

Generate a linear standard curve by plotting the average absorbance on the vertical axis versus the corresponding p53 standard concentration on the horizontal axis. The amount of p53 in each sample is determined by extrapolating OD values to p53 concentrations using the standard curve.



Typical p53 standard curve ranging from 3.12 to 100 U/mL

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 100 U/ml standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples (> 100 U/ml) have to be diluted with standard diluent or with your own sample buffer. During analysis, multiply results by the appropriate dilution factor.
The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) has not been investigated. The rate of degradation of native p53 in various matrices has not been investigated.

PERFORMANCES AND CHARACTERISTICS

Sensitivity

The minimum detectable dose of p53 is less than 1.5 U/ml.
This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 32 times.

Precision

	Intra-Assay				Inter-Assay				
Sample	**n**	**Mean (U/mL)**	**SD**	**CV%**	**Sample**	**n**	**Mean (U/mL)**	**SD**	**CV%**
A	8	53.6	1.39	2.6 %	A	5	29.1	2.0	6.8
B	8	14.7	0.62	4.2 %	B	4	5.87	0.55	9.3

ASSAY PROCEDURE SUMMARY **Total procedure length : 3h45mn**

Add 100µl of sample or diluted standard
or control

⇓

Incubate 2 hours at room temperature

⇓

Wash three times

⇓

Add 50µl of biotinylated detection antibody

⇓

Incubate 1 hour at room temperature

⇓

Wash three times

⇓

Add 100µl of Streptavidin-HRP

⇓

Incubate 30min at room temperature

⇓

Wash three times

⇓

Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for 15-20 mn.

⇓

Add 100 H_2SO_4

⇓

Read Absorbance at 450 nm