



Human P53 ELISA Kit

96 Wells

Catalogue Number: NE01P0041

FOR RESEARCH USE ONLY!

NOT FOR CLINICAL OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!



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1. BACKGROUND

P53, also designated TP53, is a DNA-binding protein of the P53 protein family, with an apparent molecular weight of approximately 53kDa as determined by protein electrophoresis. As a tumor suppressor, P53 functions across numerous cancer types to trigger growth arrest or apoptosis, with its specific biological outcome dictated by physiological context and cell type.

P53 is constitutively expressed at low basal levels in normal cells and becomes activated upon homeostatic perturbation, including DNA damage, spindle dysfunction, nutrient deprivation, heat shock, viral infection, pH shifts, hypoxia and oncogene activation. Activation of P53 initiates cell cycle arrest followed by DNA repair or apoptotic signaling, a process mediated by phosphorylation at Ser15 and Ser37 via kinases including ATM, ATR, Chk1 and MAPKs as well as DNA-PK.

P53 transcriptionally regulates a broad panel of target genes that govern core tumor-suppressive pathways, such as cell cycle arrest, DNA synthesis and repair, programmed cell death and energy metabolism.

While P53 activation generally drives cellular apoptosis, loss of P53 function facilitates tumor progression. Canonically, P53 exerts tumor-suppressive effects by binding to its cognate DNA response elements and upregulating downstream genes that suppress cell proliferation and/or invasion. Mutant P53 variants, commonly detected in a wide spectrum of human malignancies, fail to recognize consensus DNA-binding motifs, resulting in complete abrogation of tumor suppressor activity. Defects in the TP53 gene represent one of the causative drivers of esophageal carcinoma, Li-Fraumeni syndrome, lung cancer and adrenocortical carcinoma.

2. PRINCIPLE OF THE ASSAY

This ELISA kit adopts the double antibody sandwich Enzyme Linked Immunosorbent Assay. The microplate has been pre-coated with a capture antibody. The antigen to be measured and the HRP-conjugated detection antibody are then added to the microplate wells sequentially to form a capture antibody-antigen-detection antibody complex. After washing, the conjugates uninvolved in the reaction are removed, and then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP and finally turns yellow immediately after adding the stop solution. The color of the TMB substrate positively correlated with the antigen



bound in the initial steps. Measure the absorbance (OD value) at 450nm using a microplate reader, and create a standard curve along with the corresponding concentrations. Then, by inputting the OD values of the samples into the standard curve equation, calculate the concentration of the target protein in the sample.

3. KITS' ADVANCEMENT

- 1) High Specificity: Capture antibody and detection antibody are monoclonal antibodies, which respectively identify different epitopes of the antigen and maximizes the specificity of the reaction. No cross-reactivity among homologous analytes.
- 2) High Stability: The experiment uses high-quality capture antibodies and antigens, and also applies the broad-spectrum protein stabilizers, and antibody pre-coated microplate treatment to increase the thermostability of the microplate, and reproducibility of results.

4. MATERIALS (Note: Store at 2-8°C)

	REAGENTS	SPECIFICATION	QUANTITY
1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -1μg/mL	20μL	1 vial
3	HRP-conjugated Detection antibody (100×)	150μL	1 vial
4	TMB Substrates	10mL	1 vial (Avoid Light)
5	Stop Solution	10mL	1 vial
6	Wash Solution (100×)	10mL	1 vial
7	Diluent Buffer (10×)	10mL	1 vial
8	Plate Sealer		4 pieces
9	Instruction Manual		1

5. EQUIPMENT REQUIRED BUT NOT PROVIDED

- 1) 10-1000μL Pipettor
- 2) Multichannel Pipettor
- 3) 1L Sterilized Deionized Water or Ultrapure Water
- 4) Sterilized EP tubes



- 5) Absorbent Paper
- 6) Microplate Reader
- 7) High-speed Centrifuge
- 8) Microplate Washer or Washing Bottles
- 9) Incubator or Water Bath (Optional)
- 10) Data Analysis and Graphing Software
- 11) Preparation of PBS (pH 7.4): NaH_2PO_4 0.2g, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 2.9g, NaCl 8g, KCl 0.2g. Then dilute these to 1000mL with distilled water.

6. PREPARATION BEFORE ASSAY

- 1) Please read the kit instruction manual carefully before use. All reactions are performed at room temperature (20-25°C; the same applies hereinafter).
- 2) Sample Preparation: The frozen samples should be slowly restored to room temperature and then mix well, centrifuge the samples at 12000 rpm for 1 minute. Collect the supernatant for immediate assay.
- 3) Sample Dilution: Please predict the concentration before assaying. If the predicted concentration of your sample exceeds the maximum detection limit of this kit, it is recommended to dilute the sample with PBS. For the initial assay, multiple dilution factors are recommended, with serial 10-fold dilutions between adjacent concentrations.

NOTE:

- ◆ If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- ◆ Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples, and lead to inaccurate results.
- ◆ The optimal pH value of the sample is between 7.0-7.4).

7. REAGENT PREPARATION

- 1) Place all kits' components at room temperature for 30 minutes before using.
- 2) The preparation of Wash Solution (1×): Add the contents of 10mL of Wash Solution (100×) into 990mL of deionized water or ultrapure water to prepare 1000mL of Wash Solution (1×). If



crystals have formed in the concentrate, allow it to reach room temperature and mix gently until the crystals are completely dissolved.

- 3) The preparation of Diluent Buffer (1×): Dilute 10mL of Diluent Buffer (10×) with 90mL of deionized water or ultrapure water to prepare 100mL of Diluent Buffer (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The buffer is used as the Diluent Buffer for standards, samples and detection antibodies.

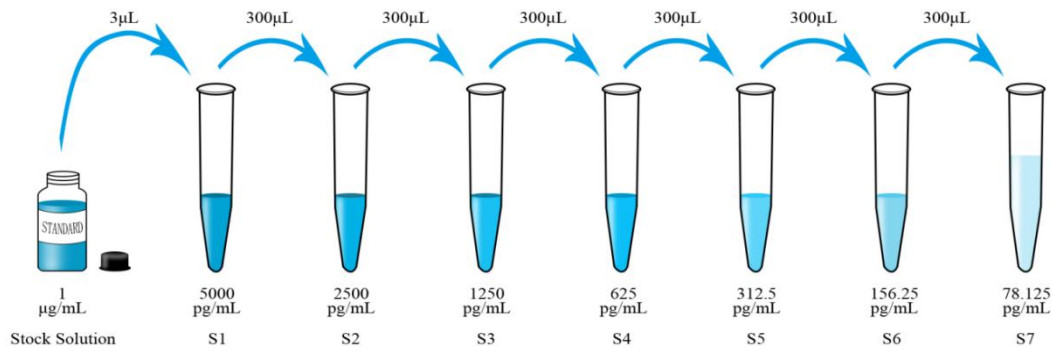
8. ASSAY PROCEDURES

- 1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean microplate frame, and start the experiment after the strips returned to room temperature (Note: The microplate frame can be reused). The standard may stick to the tube wall due to transport turbulence. Before using, gently shake it or centrifuge for about 2 seconds.
- 2) Prepare Standards: When preparing the standards, label 7 tubes, S1-S7 and add a certain volume of Diluent Buffer (1×): S1 (597μL), S2 to S7 (each 300μL).
 - ① Add 3μL of Standard (Stock Solution - 1μg/mL) into the S1. Mix gently by pipetting up and down twice, then invert to mix thoroughly.

(All subsequent mixing steps should be performed in the same manner—do not vortex or use any vigorous mixing method.)

- ② Transfer 300μL of the resulting S1 (5000pg/mL) to the tube S2, then transfer 300μL of the resulting S2 solution (2500pg/mL) to S3, mix gently. Continue this 2-fold serial dilution sequentially through S7 to achieve a final concentration of 78.125pg/mL.

NOTE: There are 7 points of diluted standards, S1: 5000pg/mL, S2: 2500pg/mL, S3: 1250pg/mL, S4: 625pg/mL, S5: 312.5pg/mL, S6: 156.25pg/mL, S7: 78.125pg/mL.



3) Sample addition: Add 100µL of standards and samples to be tested into the microplate wells. For the blank control, add only 100µL of sample diluent.

4) Cover the plate with a plate sealer, put it on the oscillator to mix and incubate for 90 minutes at room temperature.

5) Automated Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no no moisture appears.
- Fill each well with 300µL Wash Solution (1×) by a multi-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times.

Note: There should be no moisture appears in the fifth washing step.

6) Prepare HRP-conjugated Detection antibody (1×): Extract 100µL of Detection antibody (100×), and add it into 10mL Diluent Buffer to reach its working concentration (1×) and mix gently. Add 100µL of above diluted detection antibody to each well, cover the plate with a sealer, and put it



on the oscillator to mix and incubate for 90 minutes at room temperature.

- 7) Washing Step: Repeat the same procedure as step 5.
- 8) Add 100 μ L TMB substrate to each well. Cover the plate with a sealer, incubate at room temperature for about 15 minutes. If the color is light, the reaction time can be extended appropriately, but not more than 30 minutes.
- 9) Add 50 μ L of Stop Solution to each well to stop the reaction.
- 10) Run the microplate reader and conduct measurement at 450nm.
- 11) Data analysis: Four parameter curve fitting is recommended.

9. NOTES

1) Sample Preparation

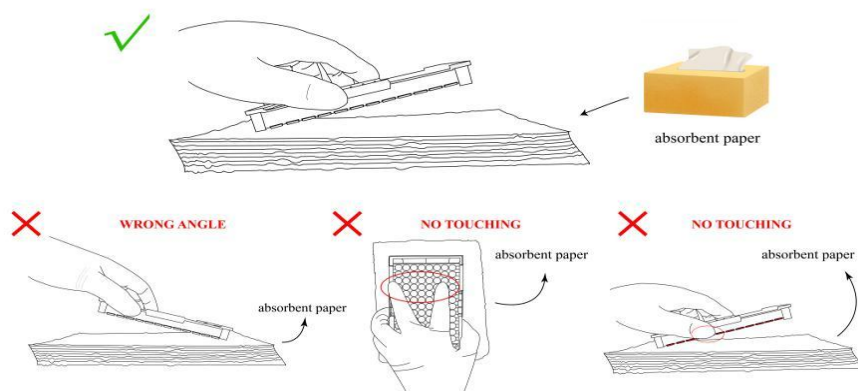
- a) After collection, the samples should be aliquoted and stored at -20°C (less than 3 months) or -80°C (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8°C.
- b) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.
- c) Certain chemical lysis buffers (e.g., SDS, Triton, etc.) may interfere with the assay and should be used with caution.
- d) It is recommended that all standards, controls and samples be run in duplicate. Gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.
- e) Do not use hyperlipidemia or hemolysis samples, which may interfere with ELISA and lead to inaccurate results.

2) Experimental Operation

- a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.
- b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.
- c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10

minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

- d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash 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. Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.
- e) TMB is easily contaminated. please protect it from light and metal.
- f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.
- g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.
- h) The coefficient of determination of the standard curve should be $R^2 \geq 0.95$.
- i) Schematic diagram of tapping the plate:



10. CALCULATION OF RESULTS

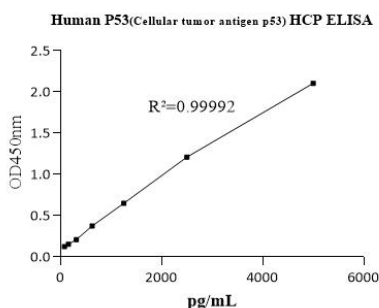
- 1) Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
- 2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four parameter logistic



(4-PL) curve-fit.

- 3) Calculate the concentration of Insulin in the samples corresponding to the mean absorbance from the standard curve.
- 4) Standard curve for demonstration only.

APPENDIX 1: EXAMPLE OF STANDARD CURVE



	Con pg/mL	OD450
S1	5000	2.106
S2	2500	1.211
S3	1250	0.652
S4	625	0.376
S5	312.5	0.212
S6	156.25	0.156
S7	78.125	0.126

11. QUALITY CONTROL

- 1) Sensitivity:

LOD: 2.4pg/mL

LOQ: 19.53pg/mL

- 2) Intra Variation%: <10%

Inter Variation%: <12%

12. FAQs & TROUBLESHOOTING

If the experimental results are abnormal, promptly take photos to record the color development results, fully retain the unused strips and reagents, and contact technical support. In addition, you may refer to the troubleshooting information provided below to identify the root cause of the problem.

Problem Description	Possible Causes	Corresponding Solutions
Poor Standard Curve	Incorrect dilution of standards	Dilute the standard curve according to the specified ratio.
	Inaccurate pipetting or sample loading	Check the pipette and pipette tips.
	Incomplete washing of the microplate	Ensure the required number of washing cycles and the volume of washing solution per well.



Weak or Absent Color Development	Insufficient incubation time	Ensure adequate incubation time.
	Incorrect experimental temperature	Use the recommended incubation temperature.
	Insufficient reagent volume or missing addition	Check the pipetting and sample loading process to ensure all reagents are added in sequence with sufficient volume.
	Substrate solution not equilibrated to room temperature	Allow the TMB substrate to stand at room temperature for more than 30 minutes before color development.
Low OD Value Reading	Incorrect microplate reader settings	Verify the wavelength and filter configuration on the microplate reader. Preheat the microplate reader in advance before reading.
High Coefficient of Variation (CV)	Incorrect sample loading	Check the sample loading operation.
	Contamination on the microplate bottom	Inspect the microplate bottom for residual liquid and fingerprints.
	Foreign objects or air bubbles in wells	Confirm no foreign objects in wells before sample loading and no air bubbles after loading.
	Unsealed or incompletely sealed plate during incubation	Seal the plate with plate sealing film.
High Background Value	Incomplete washing of the microplate	Wash the plate according to the method recommended in the instruction manual.
		If using an automated plate washer, check for clogs in all liquid inlets and waste outlets.
		For manual washing, appropriately increase the number of washing cycles.
		Inadequate or missed washing will result in high background.
	Incorrect incubation time	Operate strictly in accordance with the



	or temperature	instruction manual.
	Contaminated consumables	Ensure the tubes, pipette tips and other consumables are clean.
	Contaminated washing solution	Prepare fresh washing solution.
	Contaminated substrate solution	The substrate solution is inherently colorless. Ensure the substrate is not contaminated by metal ions or oxidizing agents before use and store it away from light.
Low Sensitivity	Improper kit storage	Store all relevant reagents according to the requirements in the instruction manual.

13. CONTACT US

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