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p53 (human) ELISA Kit

(Catalog #K4829-100, 100 assays; Refer to Section V for Storage)

I. Introduction:

BioVision's p53 Enzyme-Linked Immunosorbent Assay (ELISA) Kit is an in vitro assay for the quantitative measurement of native and recombinant human p53. The p53 protein is encoded by the *TP53* gene. It is composed of 393 amino acids but its apparent molecular mass is 53 kDa. p53 binds to a DNA consensus sequence, the p53 response element, and regulates normal cell cycle events by activating transcription of genes involved either in progression through the cell cycle, or causing arrest in G1 when the genome is damaged. It acts as a pivotal suppressor of inappropriate cell proliferation. By initiating suppressive effects through induction of apoptosis, cell senescence, or transient cell-cycle arrest, p53 plays an important role in cancer suppression, developmental regulation, and aging. p53 is found in increased amounts in a wide variety of transformed and tumor cells, where its concentration is increased 5-1000 fold over the concentration in normal cells. Meanwhile, p53 is also frequently mutated or inactivated in about 60% of cancers. The p53 ELISA assay employs an antibody specific for human p53 coated on a 96-well plate. Standards and samples are pipetted into wells and p53 present in the sample is bound to the wells by immobilized antibody. Wells are washed and a biotin-labeled human p53 specific detection antibody is added. After washing away unbound detection antibody, a streptavidin HRP-conjugate is added to the wells. The wells are again washed, a TMB substrate solution is added, and color develops in proportion to the amount of bound p53. Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Sensitivity of the kit is 0.1 ng/ml and detection range is from 0.2 ng/ml to 25.6 ng/ml. Recovery within this range is 86.3%. The intra-assay reproducibility as measured by the coefficient of variation (CV) is < 8 % & inter-assay has CV < 12 %.

II. Application:

Quantitative measurement of native and recombinant human p53

III. Specificity:

Human p53

IV. Sample Type:

- Cell lysate and cell culture supernatant
- Serum and plasma

V. Kit Contents:

Components	K4829-100	Cap Code	Part No.	Storage Temp.
Plate Coated with p53 Ab	12 strips x 8 wells	-	K4829-100-1	-20
Assay Diluent	27 ml	WM	K4829-100-2	4C
Wash Buffer A (10x)	10 ml	NM	K4829-100-3	4C
Wash Buffer B (10x)	10 ml	NM/Brown	K4829-100-4	4C
Wash Buffer C (10x)	10 ml	NM/Red	K4829-100-5	4C
rh p53 Standard	3 Vials	Yellow	K4829-100-6	-20
Detection Antibody (100x)	110 µl	Blue	K4829-100-7	-20
Streptavidin-HRP Conjugate (100x)	120 µl	Green	K4829-100-8	-20
TMB Substrate	11 ml	Amber	K4829-100-9	4C
Stop Solution	11 ml	NM/Blue	K4829-100-10	4C
Plate Sealer	2	-	K4829-100-11	4C

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Absorbent paper
- Distilled or deionized water

VII. Storage Conditions and Reagent Preparation:

Kit can be used within one year if stored unopened. We recommend storing individual components at the recommended temperatures. Avoid repeated freeze-thaw cycles. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20°C.

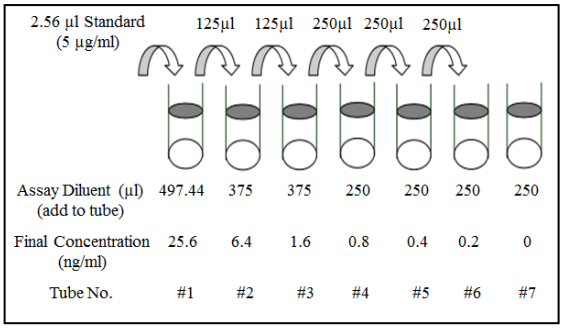
- **Wash Buffer A (10x), Wash Buffer B (10x), and Wash Buffer C (10x):** Dilute with deionized or distilled water to a final working 1x buffer concentration. If the Wash Buffers (10x) contain visible crystals, warm to room temperature and mix gently until dissolved before dilution.
- **rh p53 Standard (5 µl of 5 µg/ml Solution per vial):** Mix 2.56 µl of Standard solution (5 µg/ml) and 497.44 µl of Assay Diluent to prepare 25.6 ng/ml Standard working solution. Mix thoroughly by pipetting (**Do NOT vortex**). Unused Standard solution should be discarded. Store the Standard vials at -20°C after receiving the kit. Avoid freeze/thaw cycles.
- **Detection Antibody**: Dilute 100 fold with Assay Diluent. Use within 1 hr.
- **Streptavidin-HRP Conjugate**: Dilute only the necessary amount of HRP Conjugate 100 fold in Assay Diluent. Use within 1 hr.

VIII. Assay Protocol:

1. Bring all Buffers and desired number of Ab-coated strips to room temperature (18 - 25°C) before use. It is recommended to run all Standard dilutions in duplicate.
2. Dilute sample if required in Assay Diluent.

* Level of target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator. Suggested dilution for normal serum is 2 fold.

3. Prepare the dilution series of the Standard working solution (25.6 ng/ml) in Assay Diluent as shown in the figure. Mix each tube thoroughly by pipetting before transferring into the next tube (Do NOT vortex). Assay Diluent serves as the zero Standard (0 µg/ml).
4. Add 100 µl of each Standard and samples into appropriate wells. Cover wells with Plate Sealer and incubate for 1-1.5 hrs at 37°C. Discard the solution and wash 3 times (3 min for each wash with gentle shaking) with 200 µl of 1x Wash Solution A. After last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.



5. Add 100 µl of 1x Detection Antibody solution/well. Incubate for 1 hr at 37°C. Discard the solution. Wash 4 times with 1x Wash Solution B (3 min for each wash with gentle shaking).
6. Add 100 µl of 1x HRP Conjugate solution/well. Incubate for 45 min. to 1 hr at 37°C. Discard the solution. Wash 4 times with 1x Wash Solution C (3 min for each wash with gentle shaking).
7. Add 100 µl of TMB Substrate/well and gently shake. Measure absorbance at 650 nm for 0.5-1 min. at room temperature to monitor the blue color development, intensity of which correlates with p53 amount in samples and Standards.

   Notes:

   a. Optional: Incubation time after addition of TMB substrate must be optimized to avoid over development of color. Recommended absorbance is ~0.6-1 at 650 nm.

   b. Optional: Prepare one parallel well for background control and add TMB Substrate. Alternately, an OD_{450} value of 0.05 can be used as the background.
8. Add 100 µl of Stop Solution into each well including background control(s) and mix with gentle shaking. Remove all bubbles. Read at 450 nm within 5 min.
9. **Calculation:** Calculate the mean absorbance for each set of duplicate Standards, and subtract the reading of the background control from Standard and sample readings. Plot p53 Standard Curve. Apply corrected sample reading to the Standard Curve to get p53 concentration (ng/ml) in the sample well. If sample was diluted, multiply the value by dilution factor to calculate the concentration of human p53 in the original sample.

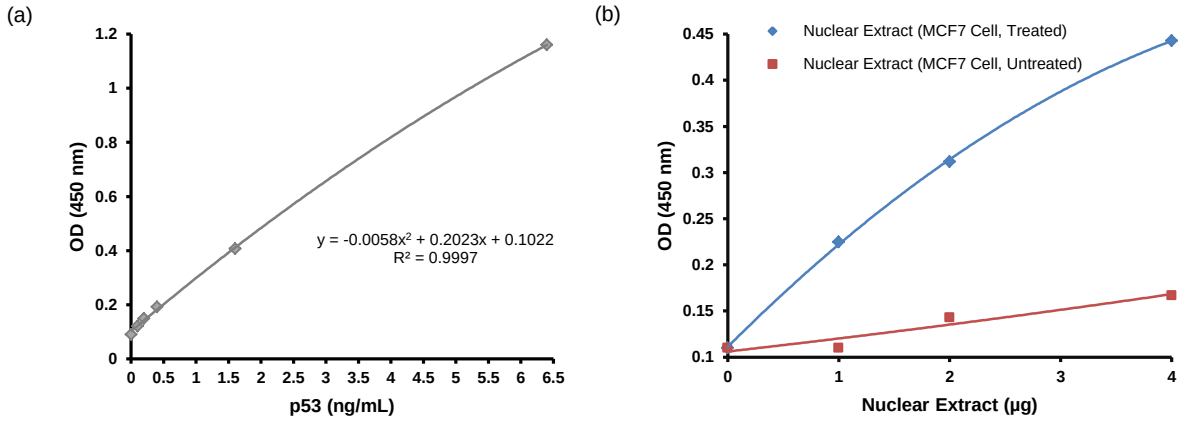


Figure: a) rh p53 Standard Curve. This standard curve is for demonstration only. The investigator is encouraged to make and select different standard concentrations to build standard curve based on different specimens. A standard curve must be run with each experiment. b) Measurement of p53 concentration in MCF7 cell nuclear extract. Cultured MCF7 cells were treated with or without H_2O_2 and the nuclear extracts were prepared by using Nuclear/Cytosol Fractionation Kit (BioVision, Catalog #K266).

RELATED PRODUCTS:

p53 Antibody (3036R-100)
p53DINP1 Antibody (3340-100)
p53DINP1 Blocking Peptide (3340BP-50)
p53R2 Antibody (3295-100)
Phospho-p53 Antibody (3515-100)
Nuclear/Cytosol Fractionation Kit (K266)
p53 Blocking Peptide (3036RBP-50)
p53 Mutant, human recombinant (4832-5)
p53, human recombinant (4829-5)

FOR RESEARCH USE ONLY! Not to be used on humans.