Human p53 ELISA Kit
(hp53-ELISA)

Cat. No. EK0895

96 Tests in 8 x 12 divisible strips

Background

p53 (also known as protein 53 or tumor protein 53), is a tumor suppressor protein that in humans is encoded by the TP53 gene. The human p53 gene is mapped to chromosome 17. Human p53 is 393 amino acids long and has seven domains. It runs as a 53-kDa protein on SDS-PAGE. The pattern of p53 splicing was specific for brain areas and for individuals. Human kidney and heart express only full-length p53. p53 plays a vital role in conserving stability by preventing genome mutation.

ScienCell's human p53 ELISA kit is based on standard sandwich enzyme-linked immune-sorbent assay technology. Human p53-specific polyclonal antibodies are pre-coated onto 8 x 12 divisible strips. The human specific detection monoclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are subsequently added to the wells and then washed with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes to yellow after adding acidic stop solution. The intensity of yellow is proportional to the amount of human p53 in the sample that is captured on the strips.

Size 96 Tests in 8 x 12 divisible strips

Assay type Sandwich ELISA

Range 156 pg/ml- 10000 pg/ml

Sensitivity < 10 pg/ml

Specificity Natural and recombinant human p53

Cross-reactivity No detectable cross-reactivity with other relevant proteins

Storage Store at 4°C for 6 months, at -20°C for 12 months.
Avoid multiple freeze-thaw cycles.

Shipping Shipped with gel ice.

Application For quantitative detection of human p53 in cell lysates.

Kit components

1. Lyophilized recombinant human p53 standard: 10 ng/tube×2.
2. 8 x 12 divisible strips pre-coated with anti- human p53 antibody.
3. Sample diluent buffer: 30 ml

4. Biotinylated anti- human P53 antibody: 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Materials Required But Not Provided

1. Microplate reader.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multi-channel pipettes are recommended for large amount of samples.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).
   Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
   Preparation of 0.01 M PBS: Add 8.5g NaCl, 1.4g Na2HPO4 and 0.2g NaH2PO4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Usage

This product is for research use only. It is not approved for use in humans, animals, or *in vitro* diagnostic procedures.

Reference

1. Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L, Benchimol S (December 1984). "Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene". EMBO J. 3 (13): 3257–62.
2. Isobe M, Emanuel BS, Givol D, Oren M, Croce CM (1986). "Localization of gene for human p53 tumour antigen to band 17p13". Nature 320 (6057): 84–5. Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C, Vogelstein B (June 1991). "Identification of p53 as a sequence-specific DNA-binding protein". Science (journal) 252 (5013): 1708–11.
3. McBride OW, Merry D, Givol D (1986). "The gene for human p53 cellular tumor antigen is located on chromosome 17 short arm (17p13)". Proc. Natl. Acad. Sci. U.S.A. 83 (1): 130–134.

Protocol for Human p53 ELISA (96-well format)

Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color developing agent should be colorless and transparent before using.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. A duplicate well assay is recommended for both standard and samples.
5. Do not let wells dry, as this will inactivate active components in wells.
6. Do not reuse tips and tubes to avoid cross contamination.
7. Avoid using reagents from different batches.

8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before use.

Preparation

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell lysates**: After sufficient splitting, there should be no obvious cell sediment .Centrifuge cell lysates at approximately 10000 X g for 5 minutes. Collect the cell lysate supernatants.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be mixed well with the diluent buffer**.

- **High target protein concentration (100-1000 ng/ml)**. The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (10-100 ng/ml)**. The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (156-10000 pg/ml)**. The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤ 156 pg/ml)**. No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the human p53 standard: p53 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of p53 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10000 pg/ml of human p53 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 minutes and mix thoroughly.
- 5000 pg/ml → 156 pg/ml of human p53 standard solutions: Label 6 Eppendorf tubes with 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10,000pg/ml P53 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human p53 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Biotinylated anti-human p53 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly (i.e. Add 1µl Biotinylated anti-human p53 antibody to 99µl antibody diluent buffer).

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly (i.e. Add 1µl ABC to 99µl ABC diluent buffer).

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard P53 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of p53 amount in samples.

1. Aliquot 0.1ml per well of the 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml human P53 standard solutions into the pre-coated 8 x 12 divisible strips. Add 0.1ml of the sample diluent buffer into the control well (**blank well**). Add 0.1ml of each properly diluted sample of human cell lysates to each empty well. See "**Sample Dilution Guideline**" above for details. We recommend that each human p53 standard solution and each sample is measured in duplicate.
2. Seal the strips with the cover and incubate at 37°C for 90 minutes.
3. Remove the cover, discard the strips' contents, and blot the strips onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human p53 antibody working solution into each well and incubate the strips at 37°C for 60 minutes.
5. Wash the strips 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 minute. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (**Strips Washing Method**: Discard the solution in the wells without touching the side walls. Blot the strips onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the strips onto paper towels or other absorbent material).
6. Add 0.1ml of prepared ABC working solution into each well and incubate the strips at 37°C for 30 minutes.
7. Wash the strips 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (See Step 5 for strip washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate the strips at 37°C in dark for 20-25 minutes (**Note**: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human p53 standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes to yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human p53 concentration of the samples can be interpolated from the standard curve.
Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the strips at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the strips at 37°C for 60 minutes. Wash strips 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the strips at 37°C for 30 minutes. Wash strips 5 times with 0.01M TBS.

4. Add TMB color developing agent and incubate the strips at 37°C in dark for 20-25 minutes.
5. Add TMB stop solution and read.

Typical Data Obtained from Human p53

(TMB reaction incubate at 37°C for 20 minutes)

Concentration (pg/ml)	0.0	156	312	625	1250	2500	5000	10,000
Absorbance (450 nm)	0.019	0.077	0.144	0.216	0.433	0.838	1.518	2.232

Typical Human p53 ELISA Kit Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.