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Antibodies | ELISA kits | Proteins

Human Phospho-P53 (Ser15) sandwich ELISA kit datasheet

For the quantitative detection of human Phospho-P53 (Ser15) in cell lysate, tissue lysate and tissue homogenate.

general information

Catalogue Number	KE40001
Product Name	Human Phospho-P53 (Ser15) Sandwich ELISA Kit
Species cross-reactivity	Human Phospho-P53 (Ser15)
sensitivity	2.3 ug/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**7157**(Human)
SwissProt	**P04637** (Human)

kit components & storage

Microplate - antibody coated 96 - well Microplate (8 well × 12 strips)	5 plates	**Unopened Kit**: Store at 2-8°C for 6 months or -20°C for 12 months **Opened Kit**: All reagents could be stored at 2-8°C for 7 days **Please use a new standard for each assay**
Phospho-P53 (Ser15) Detection antibody (100X) - 600 µL/vial	1 vial	
HRP-conjugated antibody (100X) - 600 µL/vial	1 vial	
Sample Diluent PT 4 - 150 mL/bottle	1 bottle	
Detection Diluent - 150 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 150 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 60 mL/bottle	1 bottle	
Stop Solution - 60 mL/bottle	1 bottle	
Extraction Buffer - 150 mL/bottle	1 bottle	
Plate Cover Seals	15 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 4 is for cell lysate samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

This kit do not provide positive controls. If necessary, refer to the positive control sample preparation in **example data**.

product description

KE40001 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The human Phospho-P53 (Ser15) ELISA kit is to be used to detect and quantify protein levels of endogenous Phospho-P53 (Ser15). The assay recognizes human Phospho-P53 (Ser15). An antibody specific for human P53 has been pre-coated onto the microwells. Phosphorylated sample is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for human Phospho-P53 (Ser15) is added to detect cell lysate samples. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

background

Stabilized p53 transcriptionally activates genes encoding proteins involved in cell cycle arrest, DNA repair, and/or apoptosis, and represses transcriptional activation of growth-promoting genes. Under physiological conditions, p53, in most cells, is expressed at a low or undetectable level, with a half-life of a few minutes. Under a condition of stress, such as DNA damage caused by ionizing radiation or cytotoxic agents, endogenous p53 is stabilized through a series of physiological responses, including ataxia telangiectasia mutated (ATM)/ATM and Rad3-related (ATR) activation, phosphorylation and acetylation of p53, and weakening of the binding of MDM2 to p53. Phosphorylation of serine 15 also prevents p53 from being exported from the nucleus and stimulates p53 transcriptional activity through the increased association with p300 coactivator.

sample Collection and Storage

cell lysate:

1. Collect cells and wash by centrifuging at 500xg for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.
2. Count cells and centrifuge for 5 minutes at 500xg, then discard the supernatant.
3. Add PMSF(Cat No : PR20032) and phosphatase inhibitors(Cat No: PR20015) to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.
4. Add 1 mL of Extraction reagent (containing PMSF and phosphatase inhibitors) Per 1 x 10^7 cells, Incubate cell suspension on ice for 30 minutes; be sure to invert tube containing the cell suspension several timesto complete lysis.
5. Centrifuge cell lysate at 10,000xg for 10 minutes at 4℃.
6. Measure the concentration of total protein in cell lysate using desired method of protein concentration assay. Where possible, keep samples on ice to avoid protein degradation.

tissue lysate:

1. Rinse tissue with PBS, cut into 1-2 mm pieces；
2. Add PMSF and phosphatase inhibitors to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.
3. Add 1 mL of Extraction Reagent(containing PMSF and phosphatase inhibitors) per 100 mg tissue.
4. Invert tube containing the tissue and Extraction Reagentseveral timeswhile keeping on ice for 30 minutes to ensure complete lysis.
5. Homogenize the tissue completely using desired method on ice. Centrifuge tissue homogenates at 8,000xg-10,000xg for 5minutes at 4℃. Collect the supernatant, assay immediately or aliquot and store at -20℃.
6. Measure the concentration of total protein in tissue homogenates using desired method of protein concentration assay.

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7. Avoid protein degradation by performing all the above procedures on ice where possible.

tissue Homogenates:

The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20℃. After two freeze-thawcycleswere performed to break the cell membranes, the homogenateswere centrifuged for 5 minutes at 5000xg. The supernate was removed immediately and assayed. Alternatively, aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thawcycles

sample preparation

Samples may require proper dilution to fall within the range of the assay. Cell lysate are recommended to dilute the total protein in the sample to 50-300 ug/mL.

safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.

Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	10-15 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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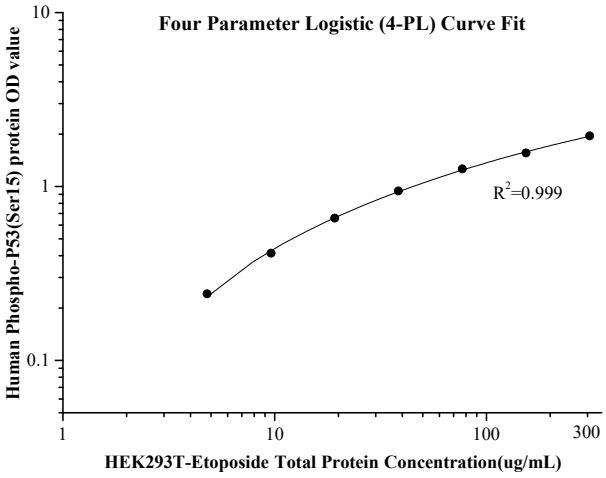
example data

These positive control curves are provided for demonstration only. A positive control curve should be generated for each set of samples assayed. The OD value of this sample used in this assay is prepared from HEK293T(30μM, 4h, from MCE, Cat: HY-136293) cell lysate treated with Etoposide.



Total protein (ug/mL)	O.D	Average	Corrected
0	0.152 0.151	0.152	-
4.8	0.390 0.395	0.393	0.241
9.6	0.568 0.561	0.565	0.413
19.2	0.814 0.804	0.809	0.658
38.4	1.094 1.092	1.093	0.942
76.9	1.418 1.406	1.412	1.261
153.8	1.707 1.713	1.710	1.559
307.5	2.103 2.102	2.103	1.951

precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

	Intra-assay Precision		Inter-assay Precision	
Sample	1	2	1	2
n	20	20	24	24
Mean (ug/mL)	150.0	20.2	149.7	19.9
SD	4.9	1.5	15.9	2.1
CV%	3.3	7.4	10.6	10.6

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sample value

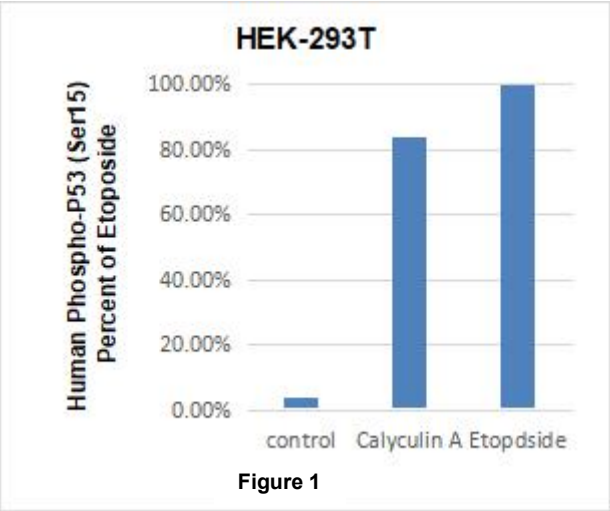

Figure 1

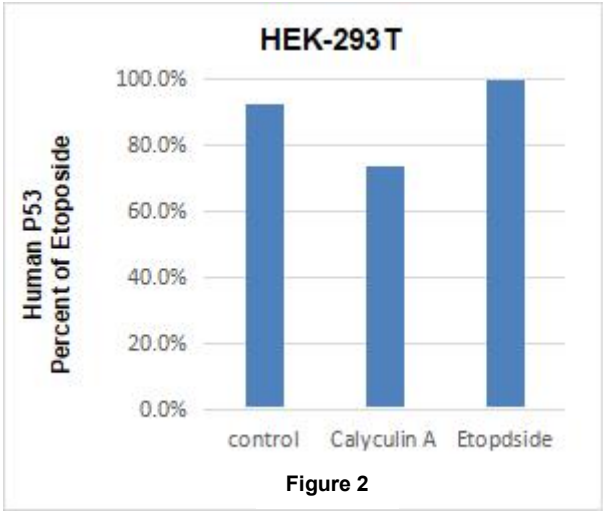

Figure 2

HEK-293T cells were cultured untreated (control) or treated with calyculin A (100nM, 0.5h, from MCE, Cat: HY-18983) or treated with etoposide (30μM, 4h, from MCE, Cat: HY-136293). An aliquot of the cell culture supernatants was removed, prepared cell lysate, analyzed by Phospho-P53 (Ser15) ELISA kit (KE40001) and human total P53 protein ELISA (KE00244). Extract of HEK-293T treated with etoposide was used for positive control sample standard curves. Relative levels interpolated from standard curves and expressed in percent of etoposide- (Figure1, Figure2) are shown.

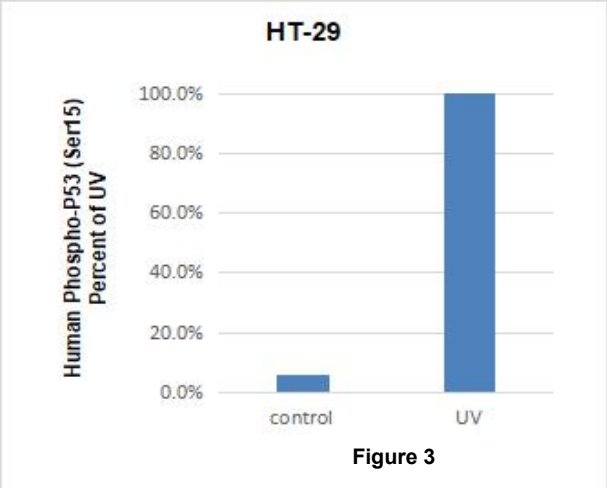

Figure 3

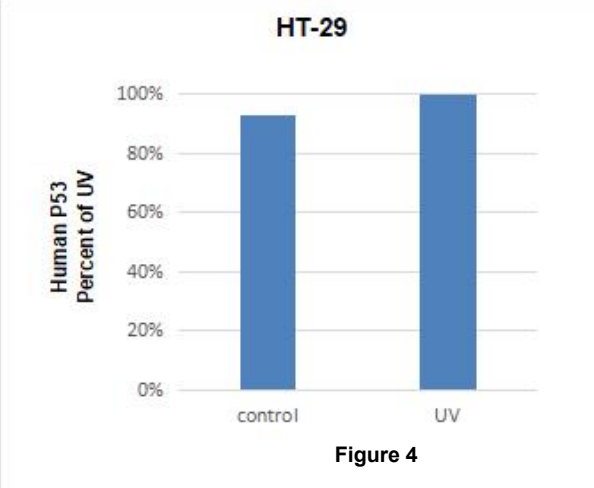

Figure 4

HT-29 cells were cultured untreated (control), treated with UV (1h). An aliquot of the cell culture supernatants was removed, prepared cell lysate, analyzed by Phospho-P53 (Ser15) ELISA kit (KE40001) and human total P53 protein ELISA (KE00244). Extract of HEK-293T treated with etoposide was used for positive control sample standard curves. Relative levels interpolated from standard curves and expressed in percent of UV- (Figure3, Figure4) are shown.

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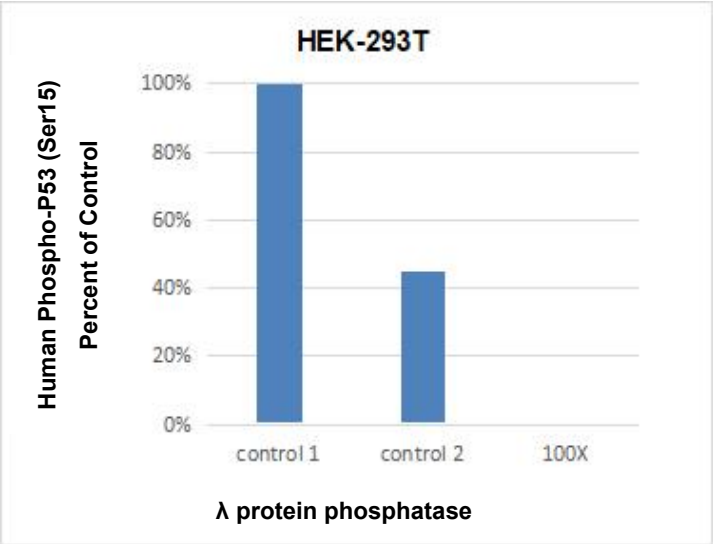

Lysates of HEK-293T cells (induced with etoposide) were treated with protease inhibitor and phosphatase inhibitors (Control 1), or left untreated (Control 2), or treated with λ protein phosphatase (100x diluted), and relative Phospho-P53 (Ser15) levels were determined using this kit. Dilutions of extracts of HEK-293T cells treated with etoposide were used to construct the standard curve.

sensitivity

The minimum detectable dose of human Phospho-P53 (Ser15) is 2.3 ug/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

references

1. Wei, Jiangbin et al. “Transcriptome profiling of cells exposed to particular and intense electromagnetic radiation emitted by the "SG-III" prototype laser facility.” Scientific reports vol. 11,1 2017. 21 Jan. 2021, doi:10.1038/s41598-021-81642-5

2. Zehbe, Ingeborg et al. “Rare human papillomavirus 16 E6 variants reveal significant oncogenic potential.” Molecular cancer vol. 10 77. 24 Jun. 2011, doi:10.1186/1476-4598-10-77

3. Zhang, Liang et al. “Activated mitochondrial apoptosis in hESCs after dissociation involving the PKA/p-p53/Bax signaling pathway.” Experimental cell research vol. 369,2 (2018): 226-233. doi:10.1016/j.yexcr.2018.05.024

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