proteintech®
Antibodies | ELISA kits | Proteins

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human P21 in cell lysate.

general information

Catalogue Number	KE00049
Product Name	P21 ELISA Kit
Species cross-reactivity	Human P21
Range (calibration Range)	0.25 - 16 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**1026** (Human)
SwissProt	**P38936** (Human)

kit components & storage

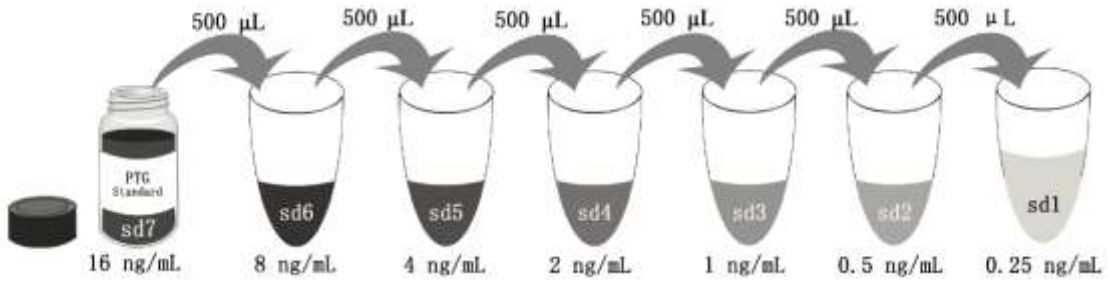
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 32 ng/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3-t - 30 mL/bottle	1 bottle	Store at -20°C, for 6 months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C, for 6 months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C, for 6 months
Extraction Reagent - 30mL/bottle	1 bottle	Store at 2-8°C, for 6 months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C, for 6 months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C, for 6 months
Plate Covers	3 pieces	

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

Sample Diluent PT 3-t is for standard and samples.

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

*Add **2 mL** Sample Diluent PT 3-t in standard. This reconstitution gives a stock solution of 16 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3-t	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00049 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The P21 ELISA kit is designed for the quantitative determination of P21 in samples of human and mouse origin. An antibody specific for P21 has been pre-coated onto the microwells. The P21 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for P21 is added to detect the captured P21 protein. 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 450nm with the correction wavelength set at 630 nm.

background

CDKN1A (p21, CIP1, WAF1), a cyclin-dependent kinase inhibitor, is necessary for proper control of the cell cycle and premature senescence. p21 is a cyclin-dependent kinase (CDK) inhibitor that suppresses proliferation by inhibiting CDK2 and CDK1 activity at the G1/S and G2/M transitions. p21 is a major mediator of p53 to induce cell cycle arrest in G1. p21 can interact with proliferating cell nuclear antigen (PCNA), and plays a regulatory role in S phase DNA replication and DNA damage repair. p21 was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation. Overexpression of p21 decreases the expression levels of cell cycle progression genes and upregulates senescence-inducing genes.

sample preparation

The cell lysates and tissue homogenates sample may require proper dilution to fall within the range of the assay. A range of dilutions, like 1:2, 1:4, 1:8 is suggested according to the individual samples. The cell lysates is better to be diluted 100 times before the assay

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

safety notes

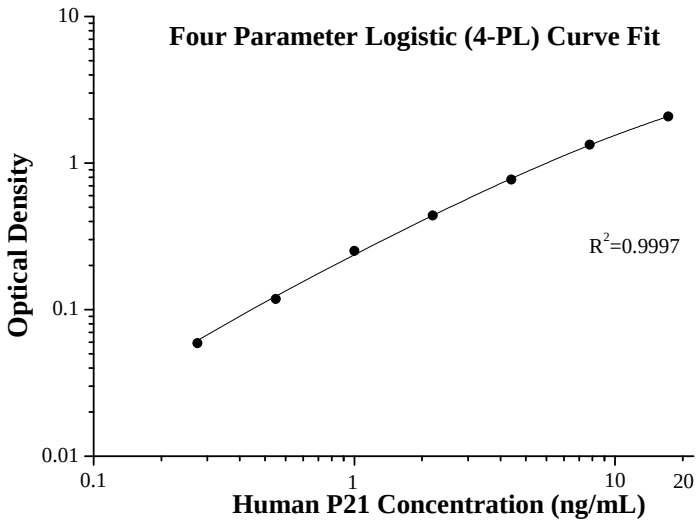
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 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	15-20 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.				

typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.028	0.0275	—
	0.027		
0.25	0.088	0.0865	0.059
	0.085		
0.5	0.147	0.1455	0.118
	0.144		
1	0.293	0.2785	0.251
	0.264		
2	0.484	0.466	0.4385
	0.448		
4	0.815	0.7995	0.772
	0.784		
8	1.39	1.3615	1.334
	1.333		
16	2.112	2.108	2.0805
	2.104		

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.

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com
ptglab.com

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/mL)	10.29	3.05	0.63	10.24	3.08	0.67
SD	0.201	0.070	0.025	0.346	0.094	0.052
CV%	2.0	2.3	4.0	3.4	3.1	7.8

recovery

The recovery of P21 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human cell lysates	1:2	83	81-86
	1:4	91	83-97

sample value

Sample Type	Concentration (ng/mL)
K562 cell lysates (1X10^{7}cell)	3.7
SHSY5Y cell lysates (1X10^{7}cell)	3.8
293 cell lysates (1X10^{7}cell)	7.4
MCF-7 cell lysates (1X10^{7}cell)	14.2

Sensitivity

The minimum detectable dose of human P21 is 0.1 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of P21 in various matrices and diluted with the appropriate **Sample Diluent PT 3-t** to produce samples with values within the dynamic range of the assay.

		Human cell lysates
1:2	Average% of Expected	89
	Range (%)	83-93
1:4	Average% of Expected	93
	Range (%)	86-97
1:8	Average% of Expected	99
	Range (%)	92-102
1:16	Average% of Expected	98
	Range (%)	90-103

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com
ptglab.com

references

1. el-Deiry WS. et al.(1994). Cancer Res. 54: 1169-74.
2. Daniel Lew. et al. (2008). Mol Biol Cell. 19: 65-77.
3. Abbas T. et al. (2009). Nat. Rev. Cancer. 9: 400-414.
4. Chang BD. et al. (2002). Proc. Natl. Acad. Sci. U. S. A. 99: 389-394.
5. provided by RefSeq, Jul 2008.

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com