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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human HSPD1 in serum and plasma.

general information

Catalogue Number	KE00032
Product Name	HSPD1 ELISA Kit
Species cross-reactivity	Human HSPD1
Range (calibration Range)	31.25 - 2000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**3329** (Human)
SwissProt	**P10809** (Human)

kit components & storage

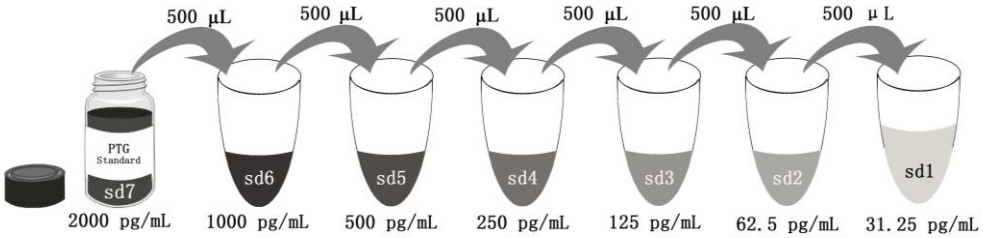
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 4000 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-af - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

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

Sample Diluent PT 1-af is for Standard and Samples.

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

*Add 2 mL Sample Diluent PT 1-af in Standard, This reconstitution gives a stock solution of 2000 pg/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 1-af	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00032 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The HSPD1 ELISA kit is to be used to detect and quantify protein levels of endogenous HSPD1. The assay recognizes human HSPD1. A polyclonal antibody specific for HSPD1 has been pre-coated onto the microwells. The HSPD1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for HSPD1 is added to detect the captured HSPD1 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.

background

HSPD1(also known as HSP60) belongs to the chaperonin family and acts as a chaperone essential for protein folding. HSP60 is a widely expressed protein and has been connected with many aspects of cell functions including gene expression regulation, DNA replication, signal transduction, differentiation, apoptosis, and cellular senescence or immortalisation. Recently it has been reported that HSP60 was significantly up-regulated in squamous cell lung cancer.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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.

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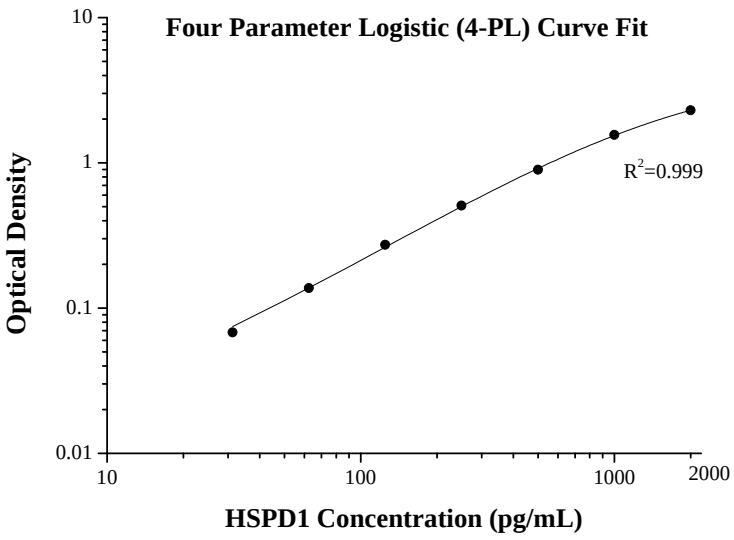
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 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.



(pg/mL)	O.D	Average	Corrected
0	0.03	0.0285	—
	0.027		
31.25	0.099	0.0965	0.068
	0.094		
62.5	0.164	0.166	0.1375
	0.168		
125	0.3	0.301	0.2725
	0.302		
250	0.517	0.537	0.5085
	0.557		
500	0.938	0.923	0.8945
	0.908		
1000	1.592	1.5825	1.554
	1.573		
2000	2.331	2.329	2.3005
	2.327		

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	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/ml)	1437.2	349.4	60.7	1462.3	352.7	61.8
SD	98.3	16.9	4.5	77.9	17.4	3.9
CV%	6.8	4.8	7.4	5.3	4.9	6.4

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recovery

The recovery of HSPD1 spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 99%, ranging from 86%-119%.

sensitivity

The minimum detectable dose of human HSPD1 is 7 pg/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 HSPD1 in human plasma and diluted with the appropriate **Sample Diluent PT 1-af** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:3)

		Citrate plasma
1:2	Average% of Expected	91
	Range(%)	79-113
1:4	Average% of Expected	88
	Range(%)	80-100
1:8	Average% of Expected	92
	Range(%)	85-101
1:16	Average% of Expected	99
	Range(%)	87-114

references

Li B, et al. Membrane proteomic analysis comparing squamous cell lung cancer tissue and tumour-adjacent normal tissue.319:118-124 (2012).

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