

## Human HSP90 Sandwich ELISA Kit Datasheet

For the quantitative detection of human HSP90 concentrations in serum, plasma, cell culture supernatants, cell lysates and urine.

### General Information

Catalogue Number	KE00054
Product Name	Human HSP90 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	125-8000 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	3320
SwissProt	P07900

### Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 16000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 µ L/vial	1 vial	
HRP-conjugated antibody (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle. For Human serum, plasma, cell culture supernatants and urine samples	1 bottle	
Sample Diluent PT 5-ef - 30 mL/bottle. For cell lysates samples	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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

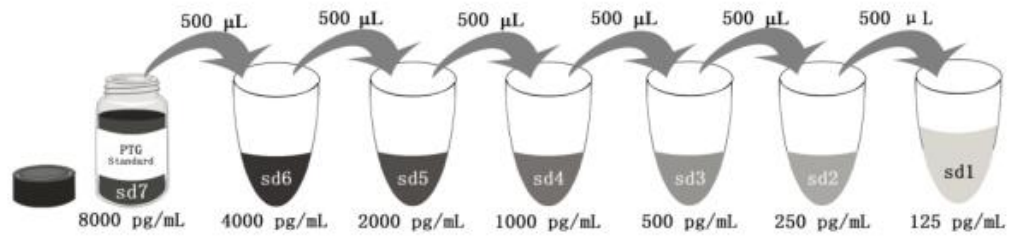
Sample Diluent PT 1-ef is for protein standard, serum, plasma, cell culture supernatants and urine.

Sample Diluent PT 5-ef is for protein standard and cell lysates.

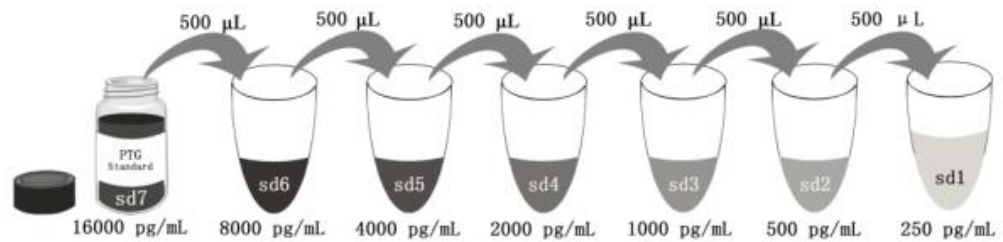
Detection Diluent is for Detection antibody and HRP-conjugated antibody.

\*Add 2 mL Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 8000 pg/mL.

\*Add 1 mL Sample Diluent PT 5-ef in protein standard. This reconstitution gives a stock solution of 16000 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-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"



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 5-ef	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00054 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The HSP90 ELISA kit is to be used to detect and quantify protein levels of endogenous HSP90. The assay recognizes human HSP90. An antibody specific for HSP90 has been pre-coated onto the microwells. The HSP90 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for HSP90 is added to detect the captured HSP90 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 450 nm with the correction wavelength set at 630 nm.

## Background

HSP90, encoded by HSP90AA1, is a constitutively and ubiquitously expressed molecular chaperone that is crucial for the stability and function of many proteins. HSP90 provides chaperoning activity for client proteins; many of them are members of oncogenic pathways, indicating its implication in tumor malignancy. HSP90 mainly resides in the cytosol, while it can also be released to the extracellular space. Secreted Hsp90 is a C-terminal truncated form. It has been reported that the level of plasma Hsp90 is positively correlated with tumor malignancy in clinical cancer patients, and can be a promising diagnostic marker for tumor malignancy in clinical application.

## 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

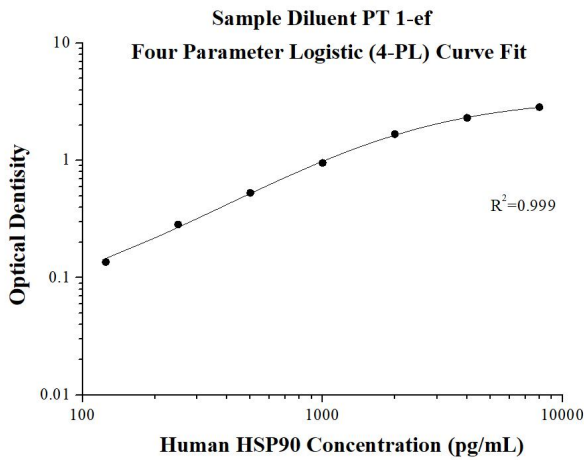
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

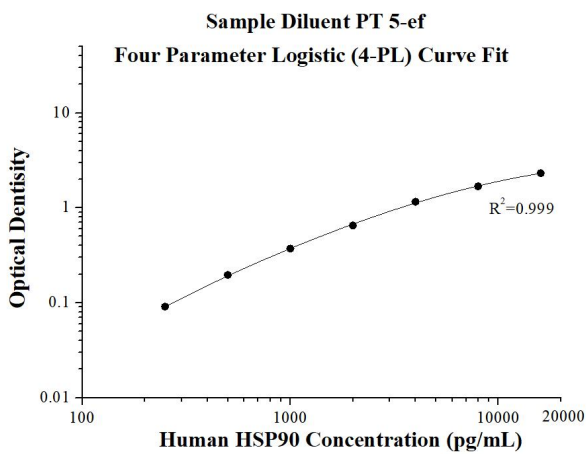
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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.				

## Example 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	0.024 0.022	0.023	-
125	0.153 0.165	0.159	0.136
250	0.291 0.317	0.307	0.284
500	0.533 0.57	0.552	0.529
1000	0.985 0.994	0.976	0.953
2000	1.626 1.777	1.702	1.679
4000	2.355 2.306	2.331	2.308
8000	2.853 2.862	2.873	2.85



(pg/mL)	O.D	Average	Corrected
0	0.027 0.027	0.027	-
250	0.118 0.117	0.118	0.091
500	0.229 0.216	0.223	0.196
1000	0.426 0.372	0.399	0.372
2000	0.685 0.672	0.678	0.651
4000	1.232 1.138	1.185	1.158
8000	1.761 1.657	1.709	1.682
16000	2.326 2.359	2.343	2.316

## 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				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	5,941.0	463.1	7.8
2	20	1,655.7	77.2	4.7
3	20	436.0	30.5	7.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	7,834.6	752.1	9.6
2	24	2,133.4	163.6	7.7
3	24	525.3	31.2	5.9

## Recovery

The recovery of HSP90 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 plasma	1:2	98	83-117
	1:4	101	82-119
Cell culture supernatants	1:2	115	101-126
	1:4	101	92-113
Urine	1:2	107	99-124
	1:4	104	81-121
Cell lysates	1:2	102	81-119
	1:4	92	80-111

## Sensitivity

The minimum detectable dose of human HSP90 is 90 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 HSP90 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

(The samples were initially diluted 1:2)

		Human plasma (Sample Diluent PT 1-ef)	Cell culture supernatants (Sample Diluent PT 1-ef)	Urine (Sample Diluent PT 1-ef)	Cell lysates (Sample Diluent PT 5-ef)
1:2	Average% of Expected	98	94	98	101
	Range (%)	90-102	92-97	88-107	99-104
1:4	Average% of Expected	101	101	100	111
	Range (%)	95-107	89-124	86-124	100-122
1:8	Average% of Expected	102	96	92	-
	Range (%)	97-103	86-114	85-98	-
1:16	Average% of Expected	105	101	93	-
	Range (%)	97-115	87-124	87-98	-

## References

1. Garcia-Carbonero R., et al. Inhibition of HSP90 molecular chaperones: moving into the clinic. *Lancet Oncol.* 14(9):e358-69 (2013).
2. Wang, X., et al. The regulatory mechanism of Hsp90 $\alpha$  secretion and its function in tumor malignancy. *Proc Natl Acad Sci U S A.* 106(50):21288-93 (2009).