

Human HSP70 Sandwich ELISA Kit Datasheet

For the quantitative detection of human HSP70 concentrations in serum, plasma, cell culture supernatants and urine.

General Information

Catalogue Number	KE00059
Product Name	Human HSP70 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	3303
SwissProt	P08107

Kit Components & Storage

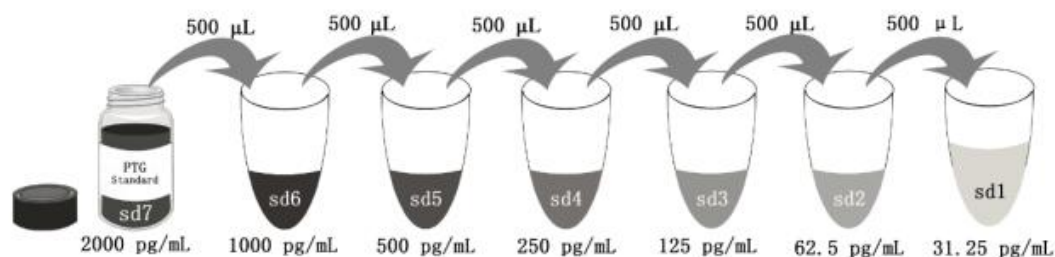
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 µ L/vial	1 vial	
HRP-conjugated antibody (100X) - 120 µ L/vial	1 vial	
Sample Diluent PT 1-ec - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 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-ec is for protein standard and samples.

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

*Add 2 mL Sample Diluent PT 1-ec in protein 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-ec	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE000059 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The HSP70 ELISA kit is to be used to detect and quantify protein levels of endogenous HSP70. The assay recognizes human HSP70. An antibody specific for HSP70 has been pre-coated onto the microwells. The HSP70 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for HSP70 is added to detect the captured HSP70 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

HSP70 is a stress-inducible member of heat-shock protein 70 (HSP70) proteins which are highly conserved chaperons implicated in protein folding, refolding, transport, and targeting. Expression of HSP70 protein can be highly activated by various stressful stimuli. Significant up-regulation of HSP70 has been found in various tumors. Increased serum HSP70 levels are also associated with progression of multiple diseases, including atherosclerosis, heart failure, and diabetes.

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

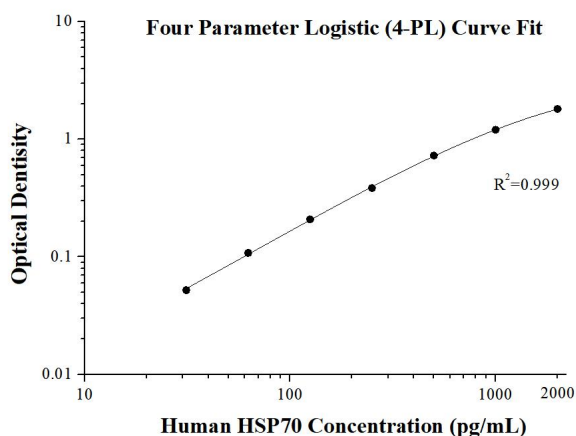
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.052 0.054	0.053	-
31.25	0.104 0.105	0.105	0.052
62.5	0.164 0.157	0.160	0.108
125	0.257 0.263	0.26	0.208
250	0.455 0.419	0.437	0.385
500	0.785 0.774	0.78	0.727
1000	1.266 1.247	1.257	1.204
2000	1.859 1.869	1.864	1.811

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	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	20	182.3	7.7	4.2	1	24	181.9	11.1	6.1
2	20	448.4	18.2	4.1	2	24	465.4	23.4	5.0
3	20	1,627.2	102.4	6.3	3	24	1,639.4	90.5	5.5

Recovery

The recovery of HSP70 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated (The samples were initially diluted 1:1).

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	104	92-124
	1:4	103	94-114
Cell culture supernatants	1:1	99	91-111
	1:2	94	80-123
Urine	1:1	84	77-92
	1:2	87	76-98

Sample Values

Serum Samples from healthy volunteers were evaluated for HSP70 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/ml)
Human serum (n=24)	369	155-758

Sensitivity

The minimum detectable dose of human HSP70 is 1.0 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 HSP70 in various matrices and diluted with the appropriate **Sample Diluent PT 1-ec** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Human plasma	Cell culture supernatants	Urine
1:2	Average% of Expected	90	108	89
	Range (%)	81-98	96-121	78-106
1:4	Average% of Expected	90	101	86
	Range (%)	82-95	93-112	85-104
1:8	Average% of Expected	95	97	96
	Range (%)	93-97	89-107	87-98
1:16	Average% of Expected	94	95	99
	Range (%)	90-97	90-102	92-103

References

1. Jenei ZM., *et al.* Elevated extracellular HSP70 (HSPA1A) level as an independent prognostic marker of mortality in patients with heart failure. *Cell Stress Chaperones*. 18(6):809-13 (2013).
2. Nakhjavani M, *et al.* Increased serum HSP70 levels are associated with the duration of diabetes. *Cell Stress Chaperones*. 15(6):959-64 (2010).