

Human Angiopoietin 1 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human Angiopoietin 1 in in serum, plasma and saliva.

General Information

Catalogue Number	KE00212
Product Name	Human Angiopoietin 1 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	62.5-4000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	284
SwissProt	Q15389

Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 8000 pg/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
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1 - 30 mL/bottle. For serum and plasma	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 4B1 - 30 mL/bottle. For saliva	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	

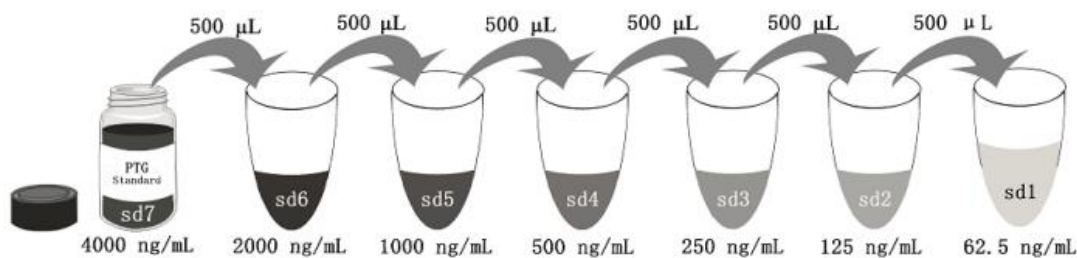
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1 is for protein standard, serum and plasma samples.

Sample Diluent PT 4B1 is for protein standard and saliva samples.

Detection Diluent is for Detection antibody and streptavidin-HRP.

*Add 2mL Sample Diluent PT 1 or PT 4B1 in protein standard. This reconstitution gives a stock solution of 4000 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 or PT 4B1	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00212 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Angiotensin 1 ELISA kit is to be used to detect and quantify protein levels of endogenous Angiotensin 1. The assay recognizes human Angiotensin 1. An antibody specific for Angiotensin 1 has been pre-coated onto the microwells. The Angiotensin 1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human Angiotensin 1 is added to detect the captured human Angiotensin 1 protein. For signal development, Streptavidin-HRP 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

Angiotensin 1 is a 70-kDa secreted glycoprotein generated from vascular mural cells, pericytes, and certain other cells. Angiotensin 1 participates in vascular differentiation through angiogenesis, which is the process of the growth and remodelling of existing vessels. Angiotensin 1 is also involved in the maintenance and turnover of blood vessels in mature animals. Angiotensin1 binds to and activates the TEK/TIE2 receptor by inducing its dimerization and tyrosine phosphorylation, playing an important role in the regulation of angiogenesis. Overexpression of Angiotensin1 has been proven to occur in malignant glioblastoma, neuroblastoma, non-small cell lung cancer and other tumors.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. 1:40 or 1:80 dilution is recommended for serum or plasma, 1:8 or 1:16 dilution is recommended for saliva samples.

Safety Notes

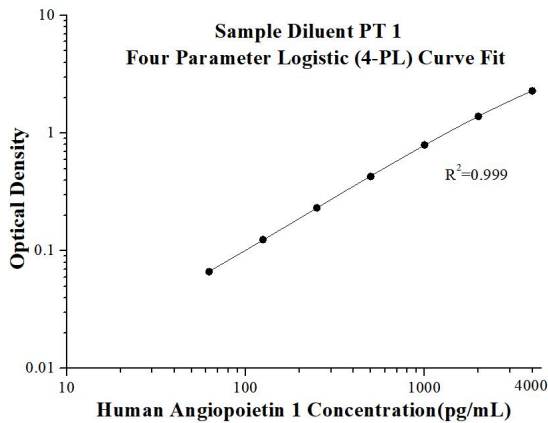
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

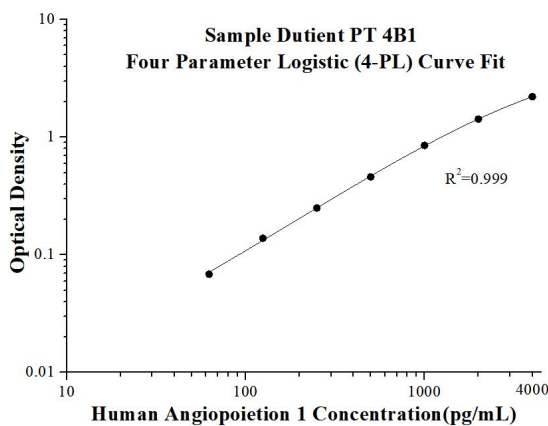
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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	0.084 0.083	0.084	-
62.5	0.152 0.148	0.150	0.067
125	0.211 0.205	0.208	0.125
250	0.318 0.313	0.316	0.232
500	0.518 0.507	0.513	0.429
1000	0.878 0.877	0.878	0.794
2000	1.475 1.472	1.474	1.390
4000	2.378 2.373	2.376	2.292



(pg/mL)	O.D	Average	Corrected
0	0.066 0.064	0.065	-
62.5	0.135 0.132	0.134	0.069
125	0.206 0.201	0.204	0.139
250	0.311 0.319	0.315	0.250
500	0.535 0.515	0.525	0.460
1000	0.895 0.937	0.916	0.851
2000	1.484 1.496	1.490	1.425
4000	2.286 2.255	2.271	2.206

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	2,021.5	94.5	4.7
2	20	557.8	13.4	2.4
3	20	155.8	6.6	4.2

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1,845.2	59.9	3.2
2	24	544.9	20.9	3.8
3	24	161.1	5.9	3.6

Recovery

The recovery of Angiopoietin 1 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 serum	1:150	92	80-99
	1:300	103	93-116
Salivas	1:30	88	71-109
	1:60	89	78-108

Sample Values

Serum, plasma and saliva samples from healthy volunteers were evaluated for Angiopoietin 1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human serum (n=15)	34,935.7	6,146.7-51,002.7

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human saliva (n=8)	4,407.2	1,721.9-13,367.6

Sensitivity

The minimum detectable dose of human Angiopoietin 1 is 17.3 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, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

(The serum samples were initially diluted 1:20. The saliva samples were initially diluted 1:4.)

		Human serum (Sample Diluent PT 1)	Saliva (Sample Diluent PT 4B1)
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	105	103
	Range (%)	104-106	102-104
1:8	Average% of Expected	112	108
	Range (%)	111-114	104-112
1:16	Average% of Expected	98	110
	Range (%)	82-110	102-119

References

1. Brooke N Horton. et al. (2010) J Histochem Cytochem.58(1):53-60.
2. C Suri. et al. (1996) Cell. 87(7):1171-80.
3. Linda J Metheny-Barlow. et al.(2003) Cell Res. 13(5):309-17.
4. Marie Jeansson. et al. (2011)J Clin Invest. 121(6):2278-89.