Human Angiopoietin 2 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human Angiopoietin 2 in cell culture supernatants.

General Information

Catalogue Number	KE00226
Product Name	Human Angiopoietin 2 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	285
SwissProt	O15123

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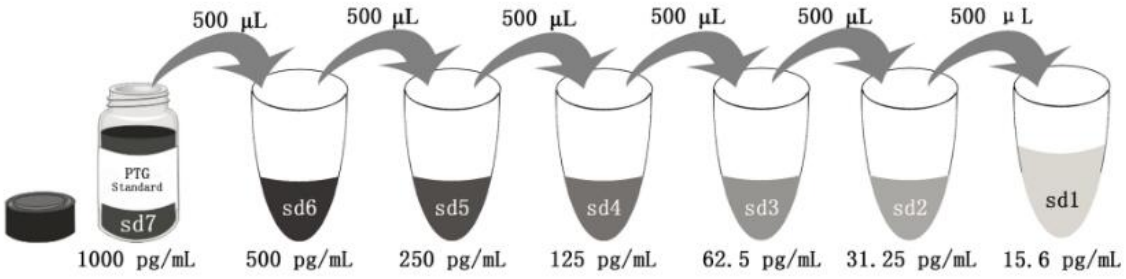
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for 6 months or -20°C 1 year
Protein standard - 1000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for 6 months or -20°C 1 year
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for 6 months or -20°C 1 year
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	Store at 2-8°C for 6 months or -20°C 1 year
Sample Diluent PT 3 - 30 mL/bottle. For cell culture supernatants.	1 bottle	Store at 2-8°C for 6 months or -20°C 1 year
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for 6 months or -20°C 1 year
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for 6 months or -20°C 1 year
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for 6 months or -20°C 1 year
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for 6 months or -20°C 1 year
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.
Sample Diluent PT 3 is for standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 3 in standard. This reconstitution gives a stock solution of 1000 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 3	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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Product Description

KE00226 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Angiopoietin 2 ELISA kit is to be used to detect and quantify protein levels of endogenous Angiopoietin 2. The assay recognizes Human Angiopoietin 2. An antibody specific for Angiopoietin 2 has been pre-coated onto the microwells. The Angiopoietin 2 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for Angiopoietin 2 is added to detect the captured Angiopoietin 2 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 450nm with the correction wavelength set at 630 nm.

Background

Angiopoietin 2 (Ang2) is a growth factor belonging to the angiopoietin/Tie (tyrosine kinase with Ig and EGF homology domains) signaling pathway, one of the main pathways involved in angiogenesis. Angiopoietin 2 was identified through a cDNA library screening, shortly after the identification of angiopoietin-1. Angiopoietin 2, is a natural antagonist for Tie2 that disrupts in vivo angiogenesis. In adult mice and humans, Angiopoietin 2 is expressed only at sites of vascular remodeling. Angiopoietin 2 has also been found to be highly expressed in diverse tumor cells and plays an important role in tumor angiogenesis and inflammation.

Sample Preparation

The samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:8, 1:16 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	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.				

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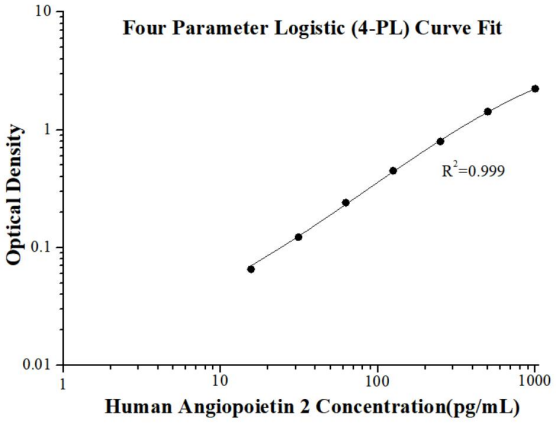
Example data

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	0.055 0.053	0.054	-
15.6	0.132 0.107	0.120	0.066
31.25	0.172 0.181	0.177	0.123
62.5	0.297 0.292	0.295	0.241
125	0.491 0.515	0.503	0.449
250	0.835 0.862	0.849	0.795
500	1.442 1.517	1.480	1.426
1000	2.294 2.274	2.284	2.230

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	32.7	1.0	3.2
2	20	122.4	4.4	3.6
3	20	495.2	20.4	4.1

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	31.6	1.3	4.1
2	24	120.9	3.6	3.0
3	24	512.1	23.5	4.6

Recovery

The recovery of Angiopoietin 2 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 (%)
Cell culture supernatants	1:32	103	81-123
	1:64	96	77-113

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Sample Values

HUVEC human umbilical vein endothelial cells were cultured in EGM-2 and grown until confluent. An aliquot of the cell culture supernate was removed, assayed for Angiopoietin 2 , and measured 4000 pg/mL.

Sensitivity

The minimum detectable dose of Human Angiopoietin 2 is 4.6 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 diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.（The samples Cell culture supernatants were initially diluted 1:4）

		Cell culture supernatants
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	104
	Range (%)	97-108
1:8	Average% of Expected	103
	Range (%)	100-106
1:16	Average% of Expected	103
	Range (%)	92-114

References

1. P C Maisonpierre. et al. (1997)Science.277(5322):55-60.
2. S Davis. et al. (1996). Cell. 87(7):1161-9.
3. Racheal G Akwii. et al. (2019) Cells. 8(5):471.
4. Christian Sfiligoi. et al. (2003)Int J Cancer. 103(4):466-74.

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