Human ZAG/AZGP1 Sandwich ELISA Kit Datasheet

For the quantitative detection of human ZAG/AZGP1 in serum and plasma samples.

General Information

Catalogue Number	KE00230
Product Name	Human ZAG/AZGP1 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	187.5-6000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	563
SwissProt	P25311

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 - 6000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1 - 30 mL/bottle	2 bottles	
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	

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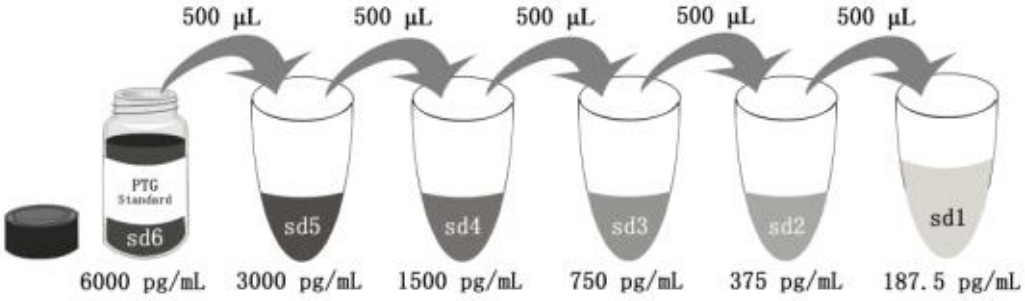
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1 is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add 1 **mL** Sample Diluent PT 1 in protein standard. This reconstitution gives a stock solution of 6000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00230 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The AZGP1 ELISA kit is to be used to detect and quantify protein levels of endogenous AZGP1. The assay recognizes human AZGP1. An antibody specific for AZGP1 has been pre-coated onto the microwells. The AZGP1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human AZGP1 is added to detect the captured human AZGP1 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

Zinc-alpha-2-glycoprotein (AZGP1) is a 41-kDa soluble protein normally found in body fluids, functions as a lipid mobilizing factor. It is synthesized by adipocytes and epithelial cells of many organs, sharing significant structural similarity with the class I major histocompatibility complex (MHC) antigens. AZGP1 plays a role in lipid metabolism, and is also involved in carcinogenesis and differentiation. Altered expression of AZGP1 has been reported in breast cancer, prostate cancer and lung adenocarcinoma, hepatocellular carcinoma, pancreatic carcinoma and oral tumors.

Sample Preparation

The samples may require proper dilution to fall within the range of the assay. 1:2000 or 1:4000 dilution is recommended for serum or plasma.

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Safety Notes

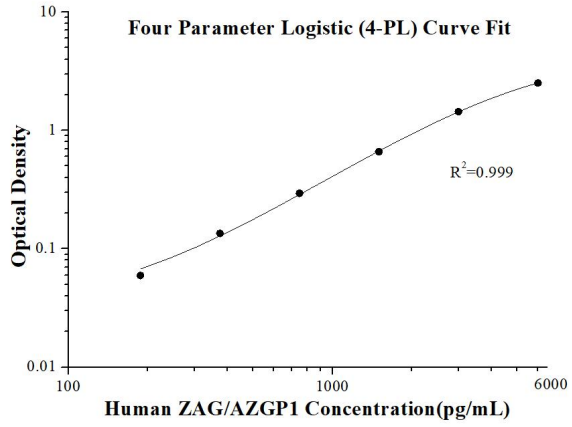
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.045 0.051	0.048	-
187.5	0.105 0.11	0.108	0.060
375	0.18 0.186	0.183	0.135
750	0.332 0.352	0.342	0.294
1500	0.694 0.721	0.708	0.660
3000	1.498 1.473	1.486	1.438
6000	2.546 2.584	2.565	2.517

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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	2,802.7	136.2	4.9
2	20	694.9	24.8	3.6
3	20	322.3	19.9	6.2

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	2,923.0	139.1	4.8
2	24	726.1	25.6	3.5
3	24	357.2	17.2	4.8

Recovery

The recovery of AZGP1 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	1:2,000	102	79-125
	1:4,000	82	79-85

Sample Values

Samples from healthy volunteers were evaluated for AZGP1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Human serum (n=16)	7,621	4,999-10,399
Human plasma (n=16)	6,048	4,524-8,294

Sensitivity

The minimum detectable dose of human AZGP1 is 13.6 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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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 1:1000)

		Human serum
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	102
	Range (%)	100-105
1:8	Average% of Expected	100
	Range (%)	97-104
1:16	Average% of Expected	89
	Range (%)	83-100

References

1. Araki T, Gejyo F, Takagaki K, et al. Complete amino acid sequence of human plasma Zn-alpha 2-glycoprotein and its homology to histocompatibility antigens. Proc Natl Acad Sci U S A. 1988;85(3):679-683.
2. Hassan MI, Waheed A, Yadav S, Singh TP, Ahmad F. Zinc alpha 2-glycoprotein: a multidisciplinary protein. Mol Cancer Res. 2008;6(6):892-906.
3. Vanni H, Kazeros A, Wang R, et al. Cigarette smoking induces overexpression of a fat-depleting gene AZGP1 in the human. Chest. 2009;135(5):1197-1208.
4. Huang Y, Li LZ, Zhang CZ, et al. Decreased expression of zinc-alpha2-glycoprotein in hepatocellular carcinoma associates with poor prognosis. J Transl Med. 2012;10:106.

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