Human A1BG Sandwich ELISA Kit Datasheet

For the quantitative detection of Human A1BG concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00130
Product Name	Human A1BG Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	125-4000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	1
SwissProt	P04217

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, biotinylated (100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-af - 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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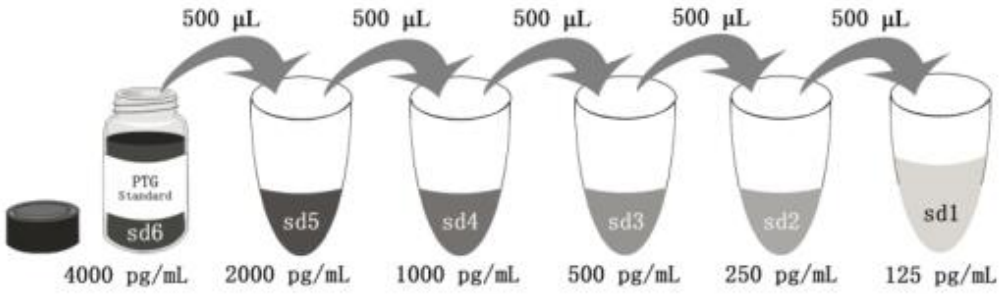
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-af is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add 1 **mL** Sample Diluent PT 1-af in protein standard. This reconstitution gives a stock solution of 4000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-af	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"

Product Description

KE00130 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The A1BG ELISA kit is to be used to detect and quantify protein levels of endogenous A1BG. The assay recognizes human A1BG. An antibody specific for A1BG has been pre-coated onto the microwells. The A1BG protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for A1BG is added to detect the captured A1BG protein. For signal development, Sterptravidin-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

Alpha-1B-glycoprotein (A1BG) is a plasma protein and is a member of the immunoglobulin superfamily. Alpha-1B-glycoprotein contains 474 amino acids and has 5 intrachain disulfide bonds. It has been reported that cysteine-rich secretory protein 3 (CRISP-3) is a specific and high-affinity ligand of alpha-1B-glycoprotein. Alpha-1B-glycoprotein-CRISP-3 complex displays a similar function in protecting the circulation from a potentially harmful effect of free CRISP3. Alpha-1B-glycoprotein is present in normal adult plasma at an average concentration of 22 mg/dl. Elevated alpha-1B-glycoprotein levels in blood have been associated with several kinds of cancer, including non-small cell lung cancer, endometrial and cervical cancer.

Sample Preparation

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The serum or plasma samples may require proper dilution to fall within the range of the assay. The human serum is better to be diluted 1:160,000 before assay, the plasma is better to be diluted 1:80,000 and 1:2 dilution is recommended for cell culture supernatants.

Safety Notes

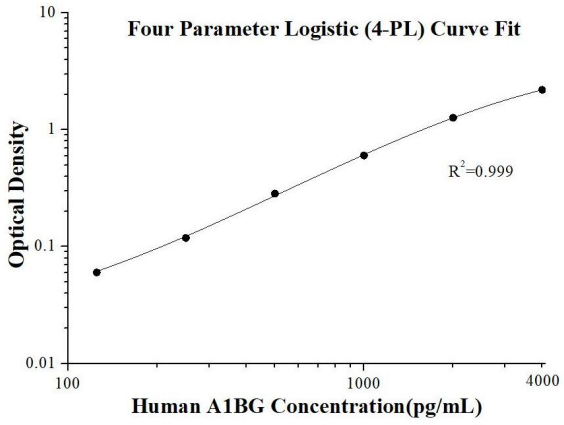
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.078 0.078	0.078	-
125	0.140 0.136	0.138	0.060
250	0.199 0.194	0.197	0.119
500	0.360 0.364	0.362	0.284
1000	0.700 0.660	0.680	0.602
2000	1.394 1.296	1.345	1.267
4000	2.284 2.256	2.272	2.193

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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	257.9	14.7	5.7
2	20	517.3	23.3	4.5
3	20	2,133.3	107.2	5.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	266.1	21.2	8.0
2	24	504.5	35.8	7.1
3	24	2,072.8	105.0	5.1

Recovery

The recovery of A1BG 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	1:80,000	101	81-122
	1:160,000	101	77-121
Human serum	1:160,000	110	99-125
	1:320,000	98	90-108
Cell culture supernatants	1:2	96	80-108
	1:4	99	80-111

Sample Values

Samples from healthy volunteers were evaluated for A1BG in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (μ g/mL)	Range (μ g/mL)
Human plasma(n=24)	132	74-560

Sensitivity

The minimum detectable dose of human A1BG is 21.5 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, cell culture supernatants were spiked with high concentrations of A1BG in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Serum and plasma samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:10,000, the serum samples were initially diluted 1:20,000)

		Human plasma	Human serum	Cell culture supernatants
1:2	Average% of Expected	100	100	80
	Range (%)	-	-	72-96
1:4	Average% of Expected	102	100	83
	Range (%)	89-110	97-103	73-94
1:8	Average% of Expected	96	106	87
	Range (%)	88-109	104-106	76-95
1:16	Average% of Expected	91	97	84
	Range (%)	84-100	97	78-96

References

1. Ishioka N, et al. Amino acid sequence of human plasma alpha 1B-glycoprotein: homology to the immunoglobulin supergene family. Proc Natl Acad Sci U S A. 83(8):2363-7 (1986).
2. Udby L, et al. Cysteine-rich secretory protein 3 is a ligand of alpha1B-glycoprotein in human plasma. Biochemistry. 43(40):12877-86 (2004).
3. Abdul-Rahman PS, et al. Expression of high-abundance proteins in sera of patients with endometrial and cervical cancers: analysis using 2-DE with silver staining and lectin detection methods. Electrophoresis. 28(12):1989-96 (2007).
4. Jeong DH, et al. Plasma proteomic analysis of patients with squamous cell carcinoma of the uterine cervix. J Gynecol Oncol. 19(3):173-80 (2008).
5. Liu Y, et al. Integrative proteomics and tissue microarray profiling indicate the association between overexpressed serum proteins and non-small cell lung cancer. PLoS One. 7(12):e51748 (2012).

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