

Human GOLM1 Sandwich ELISA Kit Datasheet

For the quantitative detection of human GOLM1 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00111
Product Name	Human GOLM1 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	0.156 - 10 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	51280
SwissProt	Q8NBJ4

Kit Components & Storage

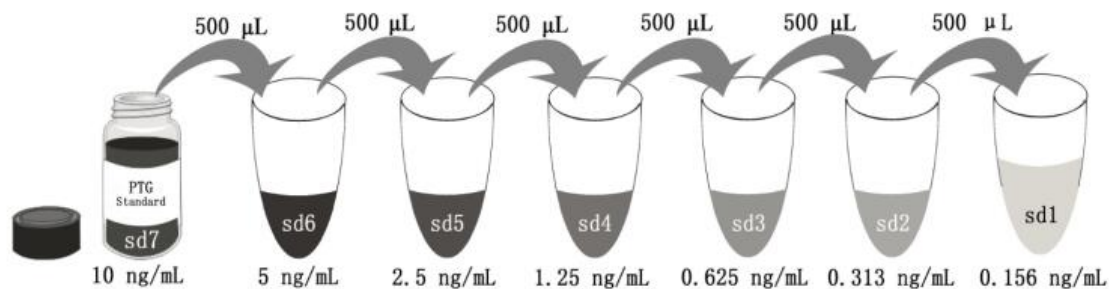
Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 20 ng/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 µ L/vial	1 vial	
HRP-conjugated (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.

*Add 2 mL Sample Diluent PT 1-ec in protein standard. This reconstitution gives a stock solution of 10 ng/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

KE00111 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The GOLM1 ELISA kit is to be used to detect and quantify protein levels of endogenous GOLM1. The assay recognizes human GOLM1. An antibody specific for GOLM1 has been pre-coated onto the microwells. The GOLM1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for GOLM1 is added to detect the captured GOLM1 protein. For signal development, 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

GOLM1 (also known as GP73 or GOLPH2) is a resident Golgi type-II membrane protein. It is predominantly expressed in the epithelial cells of many human tissues. GOLM1 traffics through endosomes and can be secreted into the circulation. Its expression is upregulated in a number of tumors and GOLM1 could be a promising serum marker for hepatocellular carcinoma.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:25 before assay, 1:2 dilution is recommended for cell culture supernatants.

Safety Notes

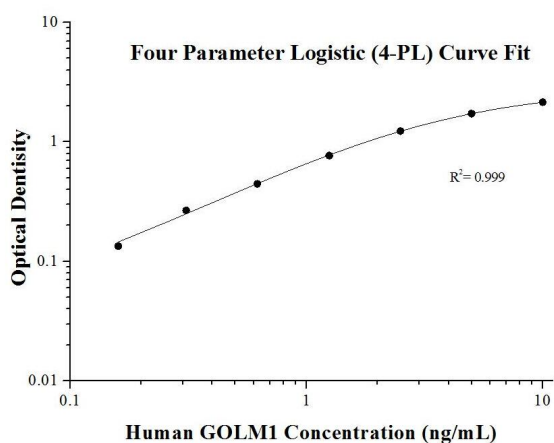
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.



(ng/mL)	O.D	Average	Corrected
0	0.081 0.08	0.0805	-
0.156	0.212 0.218	0.215	0.1345
0.313	0.335 0.361	0.348	0.2675
0.625	0.507 0.546	0.5265	0.446
1.25	0.803 0.894	0.8485	0.768
2.5	1.263 1.364	1.3135	1.233
5	1.741 1.869	1.805	1.7245
10	2.202 2.253	2.2275	2.147

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 (ng/mL)	SD	CV%
1	20	4.04	0.18	4.5
2	20	1.00	0.04	3.6
3	20	0.25	0.01	2.5

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	3.91	0.19	4.8
2	24	0.96	0.04	3.8
3	24	0.23	0.01	2.7

Recovery

The recovery of GOLM1 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	1:50	90	77-114
	1:100	99	78-112
Human serum	1:50	103	94-125
	1:100	105	86-126
Cell culture supernatants	1:2	101	78-117
	1:4	103	80-118

Sample Values

Serum and plasma samples from healthy volunteers (human) were evaluated for GOLM1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=16)	12.7	0.6-28.3
Human plasma (n=16)	7.0	1.5-25.1

Sensitivity

The minimum detectable dose of human GOLM1 is 0.01 ng/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, human plasma and serum samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture supernatants were spiked with high concentrations of GOLM1 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The serum and plasma samples were initially diluted 1:12.5)

		Human Serum	Human plasma	Cell culture supernatants
1:2	Average% of Expected	100	100	85
	Range (%)	-	-	74-96
1:4	Average% of Expected	111	113	81
	Range (%)	106-114	107-124	75-87
1:8	Average% of Expected	118	107	78
	Range (%)	111-124	85-125	77-80
1:16	Average% of Expected	98	94	80
	Range (%)	88-103	76-112	78-83

References

1. Li W. et al. (2012). Urology. 80(4):952.e1-7.
2. Riener MO. et al. (2009). Hepatology. 49(5):1602-9.
3. Chen MH. et al. (2013). Ann Surg Oncol. Suppl 3:S616-24.