Human GMFG Sandwich ELISA Kit Datasheet

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

General Information

Catalogue Number	KE00087
Product Name	Human GMFG Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	39-2500 pg/mL
Tested applications	Quantification ELISA

Database Links

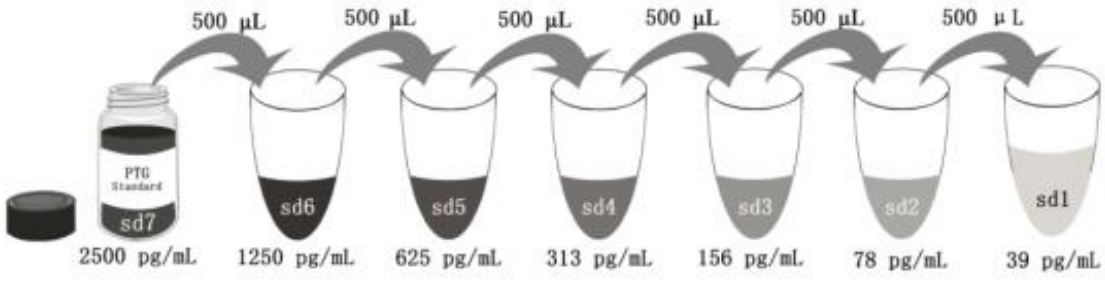
Entrez Gene	9535
SwissProt	O60234

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 - 5000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-ef - 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	

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

NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 2500 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-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00087 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The GMFG ELISA kit is to be used to detect and quantify protein levels of endogenous GMFG. The assay recognizes human GMFG. An antibody specific for GMFG has been pre-coated onto the microwells. The GMFG protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for GMFG is added to detect the captured GMFG protein. For signal development,horseradish peroxidase (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

GMFG (glia maturation factor-γ), is a highly conserved brain-specific protein that belongs to the GMF subfamily of the larger Actin-binding protein ADF family. GMFG is preferentially expressed in blood vessel cells and immune cells and its ectopic expression enhances cellular functions such as tube-formation and migration in vitro. GMFG mediates neutrophil and T-lymphocyte migration via regulation of actin cytoskeletal reorganization. GMFG also acts as an intracellular regulator of stress-activated signal transduction by demonstrating activation of p38 MAP kinase and transcription factor NF-kB in astrocytes.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4, 1:8, is suggested according to the individual samples.

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

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

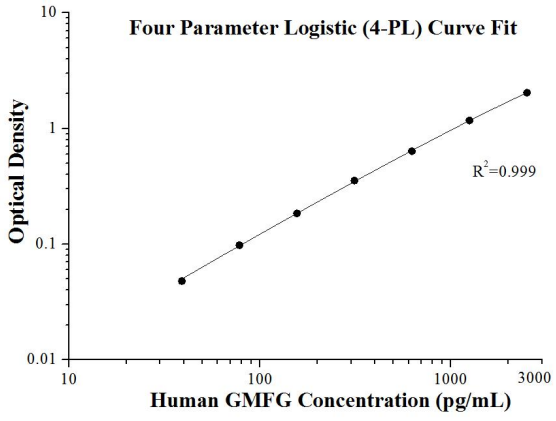
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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.				

Assay Procedure Description

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4, 1:8, is suggested according to the individual samples.

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	0.069 0.060	0.065	-
39	0.118 0.107	0.113	0.048
78	0.161 0.164	0.163	0.098
156	0.258 0.240	0.249	0.185
313	0.435 0.403	0.419	0.355
625	0.703 0.699	0.701	0.637
1250	1.258 1.215	1.237	1.172
2500	2.123 2.075	2.099	2.035

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

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	49.6	2.2	4.4
2	20	461.8	8.4	1.8
3	20	1,844.2	70.9	3.9

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	45.6	2.6	5.7
2	24	464.3	17.1	3.7
3	24	1,851.2	29.0	1.6

Recovery

The recovery of GMFG 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	1:2	101	77-128
	1:4	91	76-114
Cell culture supernatants	1:2	93	78-104
	1:4	87	78-96

Sample Values

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

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=24)	279	156-642

Sensitivity

The minimum detectable dose of human GMFG is 13.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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

Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of GMFG in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	91	105
	Range (%)	81-115	99-111
1:4	Average% of Expected	95	103
	Range (%)	80-123	98-108
1:8	Average% of Expected	97	101
	Range (%)	94-122	96-109
1:16	Average% of Expected	99	106
	Range (%)	86-118	97-123

References

1.Ikeda K. et al.(2006). Circ Res. 99:424-33.
2.Lippert DN. et al.(2012). BMC Immunol. 13:21.
3.Aerbajinai W. et al.(2011). J Leukoc Biol. 90:529-38.
4.Lippert. et al. (2012). BMC Immunol. 13: 21.
5.Zaheer A. et al. (1998). Biochem Biophys Res Commun. 250:278-82.
6.Lim R. et al. (2000). J Neurochem.74:596-602.

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