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Antibodies | ELISA kits | Proteins

colorimetric sandwich ELISA kit datasheet

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

general information

Catalogue Number	KE00001
Product Name	GMFB ELISA Kit
Species cross-reactivity	Human GMFB
Range (calibration Range)	125 - 8000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**2764** (Human)
SwissProt	**P60983** (Human)

kit components & storage

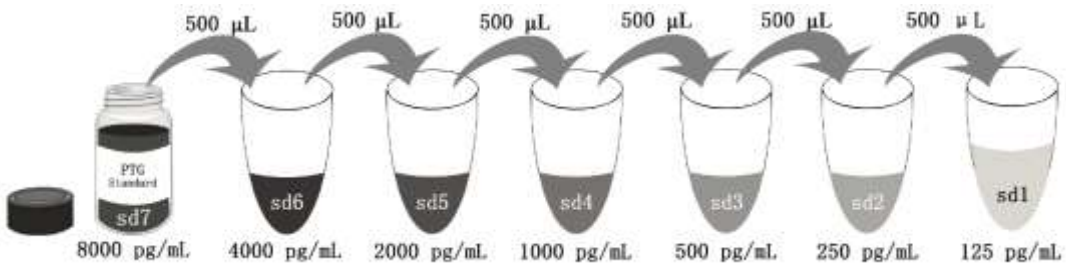
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 16000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-af - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-af is for standard and samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add **2 mL** Sample Diluent PT 1-af in standard. This reconstitution gives a stock solution of 8000 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-af	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00001 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The GMFB ELISA kit is to be used to detect and quantify protein levels of endogenous GMFB. The assay recognizes human GMFB. An antibody specific for GMFB has been pre-coated onto the microwells. The GMFB protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for GMFB is added to detect the captured GMFB 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 450nm with the correction wavelength set at 630 nm.

background

GMFB, Glia maturation factor beta, is a 17kd protein which initially identified as a nerve growth factor implicated in nervous system development. GMF-beta protein is specific to the brain where it is expressed in glial cells (mainly astrocytes) and insome neurons. GMFB causes differentiation of brain cells, stimulation of neural regeneration, and inhibition of proliferation of tumor cells. GMFB can be considered as a prognostic predictor for SOC(Serous ovarian carcinoma) patients. This kit is used to quantify GMFB level in vivo.

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 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.

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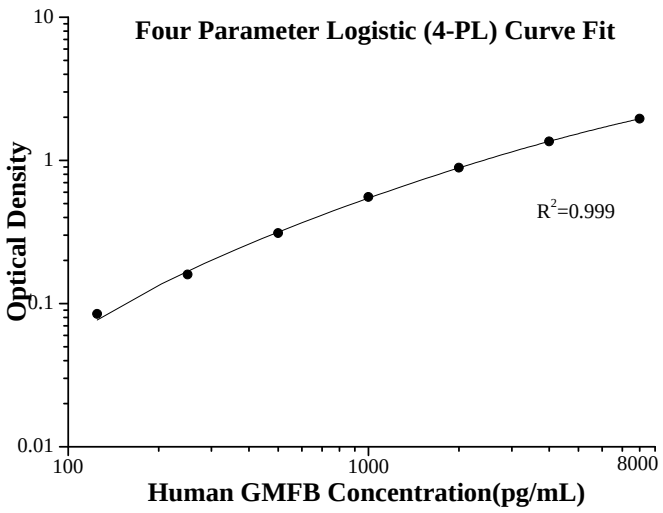
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 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.				

typical 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	0.067	0.0665	—
	0.066		
125	0.151	0.151	0.0845
	0.151		
250	0.226	0.226	0.1595
	0.226		
500	0.378	0.3765	0.31
	0.375		
1000	0.605	0.6205	0. 554
	0.636		
2000	0.921	0.9545	0.888
	0.988		
4000	1.408	1.421	1.3545
	1.434		
8000	1.969	2.021	1.9545
	2.073		

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.

Plasma Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	2737.4	1313.3	458.9	3655.0	1700.1	652.2
SD	199.3	73.7	36.1	431.4	146.8	37.4
CV%	7.3	5.6	7.9	11.8	8.6	5.7

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Cell Culture Supernatants Assay

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	6,361.0	2,641.6	1,467.3	5,734.9	2,553.0	1,509.7
SD	413.5	143.3	102.0	753.2	164.0	83.0
CV%	6.5	5.4	7.0	13.1	6.4	5.5

recovery

The recovery of GMFB 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	87	75-102
Cell culture supernatants	91	83-99

sensitivity

The minimum detectable dose of human GMFB is 23.8 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 spiked with high concentrations of GMFB in various matrices and diluted with the appropriate **Sample Diluent PT 1-af** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:2)

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	71	104
	Range (%)	69-73	97-111
1:4	Average% of Expected	85	103
	Range (%)	75-94	97-113
1:8	Average% of Expected	95	105
	Range (%)	88-101	96-120
1:16	Average% of Expected	102	108
	Range (%)	101-103	91-125

references

1. Kaplan, R., et al. Molecular cloning and expression of biologically active human glia maturation factor-beta. J. Neurochem. 57: 483-490(1991).
2. Yan Li Li, et al. Identification of glia maturation factor beta as an independent prognostic predictor for serous ovarian cancer. Eur J Cancer. ;46(11):2104-18(2010).

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