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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human GM-CSF in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00003
Product Name	GM-CSF ELISA Kit
Species cross-reactivity	Human GM-CSF
Range (calibration Range)	3.9 - 250 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**1437** (Human)
SwissProt	**P04141** (Human)

kit components & storage

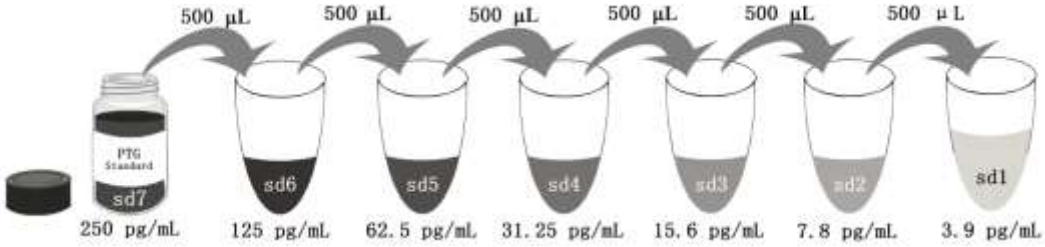
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 500 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) , biotinylated - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 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-ef is for standard and samples.

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

*Add **2 mL** Sample Diluent PT 1-ef in standard. This reconstitution gives a stock solution of 250 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

KE00003 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The GM-CSF ELISA kit is to be used to detect and quantify protein levels of endogenous GM-CSF. The assay recognizes human GM-CSF. An antibody specific for GM-CSF has been pre-coated onto the microwells. The GM-CSF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for GM-CSF is added to detect the captured GM-CSF 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 450nm with the correction wavelength set at 630 nm.

background

CSF2, also named as GM-CSF, is an important hematopoietic growth factor and immune modulator, which is produced by a variety of cell types including T cells, macrophages, endothelial cells and fibroblasts upon receiving immune stimuli. It was originally recognized as a stimulator for the proliferation of granulocytes and macrophages from bone marrow precursor cells. It has also been shown to promote the survival and activation of mature myeloid cells and therefore contributes to the maintenance of innate immune homeostasis. Recent studies suggest that GM-CSF also has proinflammatory functions and plays critical roles in the development of autoimmune and inflammatory diseases, particularly in Th17 driven diseases. GM-CSF also plays a role in embryonic development by functioning as an embryokine produced by reproductive tract.

sample preparation

The serum, plasma or cell culture supernatants 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.

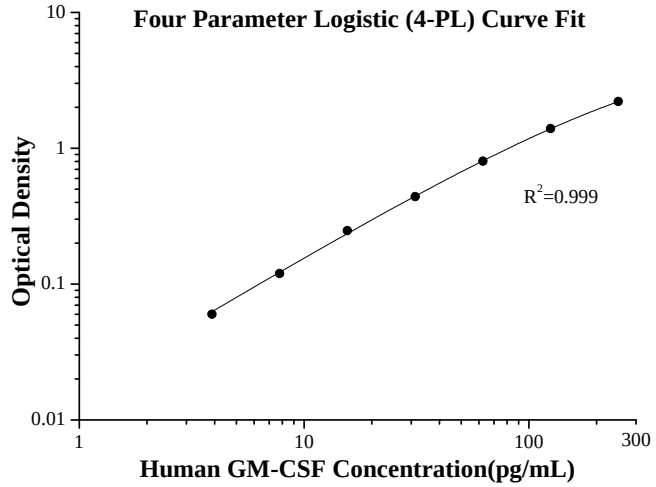
USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

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.				

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	0.023	0.0255	—
	0.028		
3.9	0.082	0.0855	0.06
	0.089		
7.8	0.143	0.145	0.1195
	0.147		
15.6	0.279	0.2725	0.247
	0.266		
31.25	0.461	0.466	0.4405
	0.471		
62.5	0.856	0.8285	0.803
	0.801		
125	1.42	1.4205	1.395
	1.421		
250	2.209	2.2295	2.204
	2.25		

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			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	206.1	40.7	5.3	272.8	72.3	11.8
SD	12.1	2.8	0.4	20.9	4.1	1.0
CV%	5.9	6.9	7.3	7.7	5.7	8.8

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ptglab.com

recovery

The recovery of GM-CSF 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 (%)
Citrate plasma	1:4	94	80-109
	1:8	92	84-98
Cell culture supernatants	1:2	98	88-107
	1:4	85	77-91

sample values

Cell culture supernates-Human peripheral blood mononuclear cells (5 x 10^5 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 µ g/mL streptomycin sulfate. Cells were stimulated with the agents listed in the table below. Aliquots of the cell culture supernate were removed on days 1, 3, and 5 and assayed for levels of human GM-CSF.

Stimulated/Unstimulated	Concentration (pg/mL)
10 ug/mL PHA for 1 day	207
10 ug/mL PHA for 1 day + 50 ng/mL LPS for 2 hours	275
Unstimulated for 1 day	72
10 ug/mL PHA for 3 days	1,077
10 ug/mL PHA for 2 days + 50 ng/mL LPS for 1 day	1,181
Unstimulated for 3 days	55
10 ug/mL PHA for 5 days	1,910
10 ug/mL PHA for 3 days + 50 ng/mL LPS for 2 days	1,339
Unstimulated for 5 days	41

sensitivity

The minimum detectable dose of human GM-CSF is 1.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of GM-CSF in various matrices and diluted with the appropriate **Sample Diluent PT 1-ef** to produce samples with values within the dynamic range of the assay. (The serum and plasma samples were initially diluted 1:1)

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		Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	91	102
	Range (%)	80-98	99-106
1:4	Average% of Expected	101	104
	Range (%)	93-110	100-107
1:8	Average% of Expected	113	103
	Range (%)	110-116	97-107
1:16	Average% of Expected	112	110
	Range (%)	110-114	101-119

references

1. Burgess AW. et al. (1977). J Biol Chem.252: 1998-2003.
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