

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of mouse G-CSF concentrations in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE10025
Product Name	G-CSF ELISA Kit
Species cross-reactivity	Mouse G-CSF
Range (calibration Range)	7.8 - 500 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	12985 (Mouse)
SwissProt	P09920 (Mouse)

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, biotinylated (100X) - 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. For serum and plasma.	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-ec - 30 mL/bottle. For cell culture supernatants.	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	

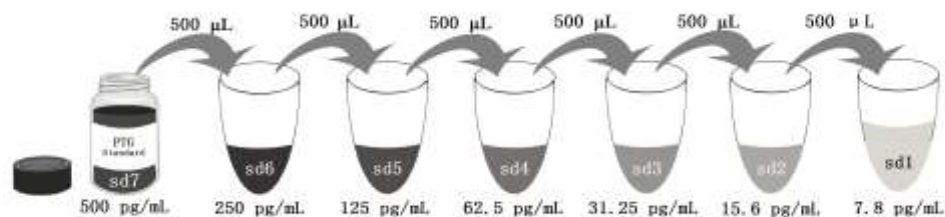
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ef is for standard, serum and plasma.

Sample Diluent PT 1-ec is for standard and cell culture supernatants.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 1-ef or PT 1-ec in standard. This reconstitution gives a stock solution of 500 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 or PT 1-ec	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE10025 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Mouse G-CSF ELISA kit is to be used to detect and quantify protein levels of endogenous mouse G-CSF. The assay recognizes mouse G-CSF. An antibody specific for mouse G-CSF has been pre-coated onto the microwells. The mouse G-CSF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse G-CSF is added to detect the captured mouse G-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

Granulocyte colony-stimulating factor (G-CSF), also referred to as CSF3, is a protective cytokine with anti-inflammatory effects. G-CSF is important in promoting survival of the granulocytic lineage cells and proliferation and migration of neutrophils as well as trophoblast cells. G-CSF acts by binding to its receptor G-CSFR (also called CSF3R), which after binding with G-CSF activates the canonical Janus kinase (Jak)/signal transducer, activator of transcription (STAT) and Ras/Raf/MAP kinase pathways. G-CSF potently stimulates the proliferation and release of peripheral blood progenitor cells into the bloodstream and is therefore used to treat neutropenia after chemotherapy. Furthermore, G-CSF levels are elevated upon intensive exercise leading to increased neutrophil counts, which are predominantly due to delayed neutrophil apoptosis.

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

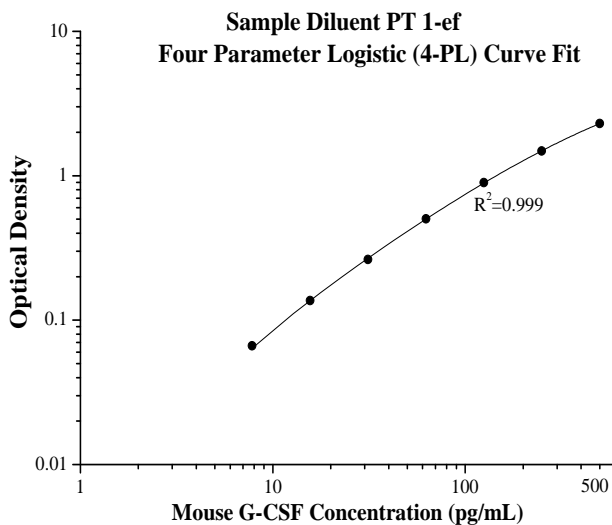
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

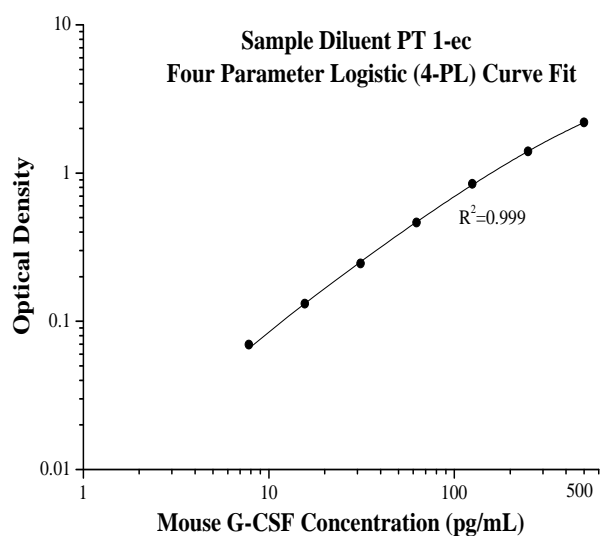
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	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	0.056	0.056	—
	0.056		
7.8	0.124	0.123	0.067
	0.121		
15.6	0.191	0.193	0.137
	0.194		
31.3	0.315	0.319	0.263
	0.323		
62.5	0.546	0.558	0.502
	0.57		
125	0.93	0.951	0.895
	0.972		
250	1.52	1.542	1.486
	1.563		
500	2.345	2.356	2.30
	2.366		



(pg/mL)	O.D	Average	Corrected
0	0.054	0.058	—
	0.062		
7.8	0.125	0.128	0.07
	0.13		
15.6	0.184	0.19	0.132
	0.195		
31.3	0.299	0.304	0.246
	0.308		
62.5	0.516	0.522	0.464
	0.528		
125	0.861	0.903	0.845
	0.944		
250	1.44	1.455	1.397
	1.47		
500	2.272	2.253	2.195
	2.233		

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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	216.2	85.3	8.8	209.1	86.2	51.2
SD	7.1	2.5	0.9	10.2	4.6	3.8
CV%	3.3	2.9	9.7	4.9	5.3	7.4

recovery

The recovery of G-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 (%)
Mouse serum	1:2	94	74-115
	1:4	96	75-127
Cell culture supernatants	1:2	109	88-124
	1:4	101	85-118

sample values

Twenty-two individual mouse serum samples were evaluated for the presence of mouse G-CSF in this assay.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Mouse serum (n=22)	266	117-745

sensitivity

The minimum detectable dose of mouse G-CSF is 0.8 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 mouse G-CSF in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Mouse serum (Sample Diluent PT 1-ef)	Cell culture supernatants (Sample Diluent PT 1-ec)
1:2	Average% of Expected	-	98
	Range (%)	-	94-103
1:4	Average% of Expected	105	106
	Range (%)	97-123	95-117
1:8	Average% of Expected	115	100
	Range (%)	106-123	89-111
1:16	Average% of Expected	123	90
	Range (%)	118-128	83-97

references

1. Panopoulos AD. et al. (2008). Cytokine. 42(3):277-88.
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3. Sheridan WP. et al.(1989). Lancet. 2(8668):891-5.
4. Sheridan WP. et al.(1992). 339(8794):640-4.
5. Yamada M. et al. (2002). 92(5):1789-94.
6. Mooren FC. Et. Al.(2012). 113(7):1082-90.