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Antibodies | ELISA kits | Proteins

AuthentiKine™

Human M-CSF Sandwich ELISA Kit Datasheet

For the quantitative detection of human M-CSF concentrations in serum, plasma, cell culture supernatants, urine and saliva.

General Information

Catalogue Number	KE00184
Product Name	AuthentiKine™ Human M-CSF Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	15.6-1000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	1435
SwissProt	P09603

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 - 2000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle. For serum, plasma samples and cell culture supernatants	1 bottle	
Sample Diluent PT 4 - 30 mL/bottle. For urine and saliva	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	

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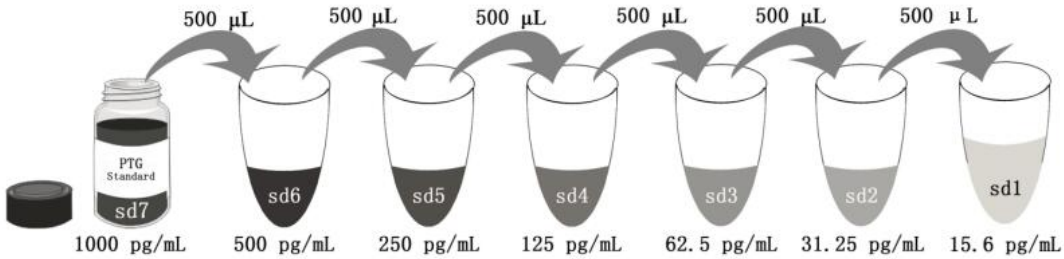
NB: Do not use the kit after the expiration date.
Sample Diluent PT **4B1** is for protein standard, serum, plasma and cell culture supernatants.
Sample Diluent PT **4** is for protein standard, urine and saliva.
Detection Diluent is for Detection antibody.
*Add **2 mL** Sample Diluent PT 4B1 or PT 4 in protein standard. This reconstitution gives a stock solution of 1000 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 4B1 or PT 4	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00184 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The M-CSF ELISA kit is to be used to detect and quantify protein levels of endogenous M-CSF. The assay recognizes human M-CSF. An antibody specific for M-CSF has been pre-coated onto the microwells. The M-CSF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for M-CSF is added to detect the captured M-CSF protein. For signal development, 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

M-CSF, also called colony-stimulating factor-1 or CSF-1), is a haematopoietic growth factor, which controls survival, proliferation, differentiation and function of mononuclear phagocytes. It also plays a role in bone metabolism, fertility, pregnancy and inflammatory processes. M-CSF may be expressed on the surface of the cell as a membrane-spanning glycoprotein or secreted into circumstance. M-CSF was identified as an 84-kDa homodimeric glycoprotein found in healthy human urine, and is present in the peripheral blood and urine. M-CSF serum levels were elevated in pre-transplant haemodialysis patients, so it might be a specific marker of Kidney Allograft Rejection.

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A minimum 1:4 or 1:8 dilution is recommended for serum or plasma. A minimum 1:2 or 1:4 dilution is recommended for cell culture supernatants, saliva, urine.

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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	Detection Antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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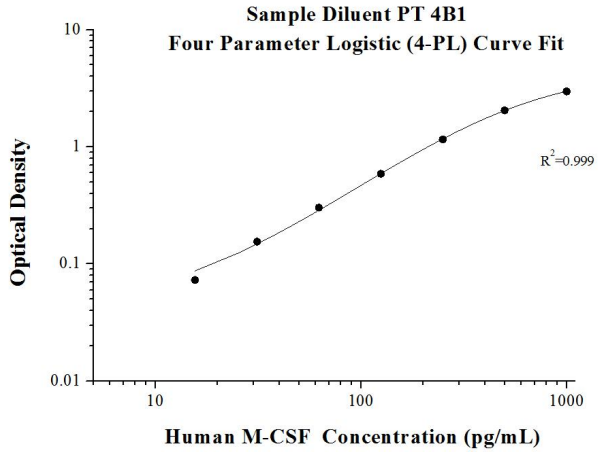
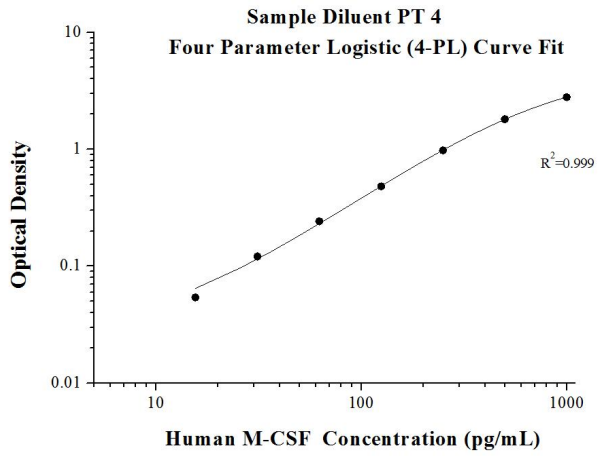
Example data

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	0.079 0.075	0.077	-
15.6	0.158 0.141	0.150	0.073
31.25	0.238 0.225	0.232	0.155
62.5	0.376 0.383	0.380	0.303
125	0.686 0.646	0.666	0.589
250	1.243 1.227	1.235	1.158
500	2.126 2.133	2.130	2.053
1000	3.08 3.029	3.055	2.978



(pg/mL)	O.D	Average	Corrected
0	0.084 0.083	0.084	-
15.6	0.139 0.136	0.138	0.054
31.25	0.205 0.205	0.205	0.121
62.5	0.323 0.329	0.326	0.242
125	0.56 0.57	0.565	0.481
250	1.065 1.057	1.061	0.977
500	1.91 1.87	1.890	1.806
1000	2.875 2.873	2.874	2.790

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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	207.3	5.5	2.7
2	20	433.8	8.8	2.0
3	20	797.3	51.7	6.5

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	214.6	6.9	3.2
2	24	475.3	22.1	4.7
3	24	880.9	48.0	5.0

Recovery

The recovery of M-CSF spiked to three different levels in four samples throughout the range of the assay in human samples were evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:4	79	71-88
	1:8	84	79-88
Cell culture supernatants	1:2	85	80-94
	1:4	85	82-89
Urine	1:8	96	89-101
	1:16	95	93-97
Saliva	1:2	89	84-93
	1:4	94	86-107

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Sample Values

Human serum, urine and saliva samples were evaluated for human M-CSF in this assay.

Sample Type	Mean(pg/mL)	Rang (pg/mL)
Human serum (n=16)	712.6	226-2,041.6
Urine (n=6)	1,272.6	216.3 – 2,082.1
Saliva (n=3)	185.5	21.3-473.0

Cell Culture supernatants - Human peripheral blood mononuclear cells (PBMC) (1 × 10^6 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin sulfate. The cell culture supernatants were stimulated with 10 ug/mL of PHA. An aliquot of the culture supernatants were removed, assayed for human M-CSF.

Stimulated conditions	Day 3 (pg/mL)	Day 5 (pg/mL)
PHA 10 ug/mL	82.8	181.6
Unstimulated	-	-

A549 human lung carcinoma cells were cultured in MEM and supplemented with 10% fetal bovine serum until confluent. An aliquot of the cell culture supernatants was removed, assayed for human M-CSF and measured 338 pg/mL.

U2OS human osteosarcoma cells were cultured in MEM and supplemented with 10% fetal bovine serum fetal bovine serum until confluent. An aliquot of the cell culture supernatants was removed, assayed for human M-CSF and measured 108 pg/mL.

Sensitivity

The minimum detectable dose of human M-CSF is 1.1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human serum (PT 4B1)	Cell culture supernatants (PT 4B1)	Urine (PT 4)	Saliva (PT 4)
1:2	Average% of Expected	100	100	100	100
	Range (%)	-	-	-	-
1:4	Average% of Expected	88	101	94	94
	Range (%)	81-97	93-110	87-100	80-118
1:8	Average% of Expected	84	105	96	
	Range (%)	82-89	96-113	84-105	
1:16	Average% of Expected	80	96	92	
	Range (%)	79-81	83-109	83-110	

Calibration

This immunoassay is calibrated against a highly purifed HEK293 cell-expressed recombinant human M-CSF produced at Proteintech Systems.

The NIBSC/WHO recombinant Human M-CSF Standard 89/512 was evaluated in this kit. The dose response curve of the NIBSC standard 89/512 parallels the standard curve. To convert sample values obtained with the Human M-CSF kit to approximate NIBSC International Units, use the equation below:

NIBSC (89/512) approximate value (IU/mL) = 0.281 x Proteintech Human M-CSF value (pg/mL)

References

1. J W Pollard. et al. (1987) Nature. 330(6147): 484-6.
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5. K Motoyoshi. et al. (1978) Blood. 52(5): 1012-20.
6. Yannick Le Meur. et al. (2004) Nephrol Dial Transplant.19(7):1862-5.

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