

## Human M-CSF sandwich ELISA kit datasheet

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

### general information

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

### database links

Entrez Gene	1435 (Human)
SwissProt	P09603 (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- 2000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 4B1- 30 mL/bottle. For serum, plasma samples and cell culture supernatants	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 4 - 30 mL/bottle. For urine and saliva	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	

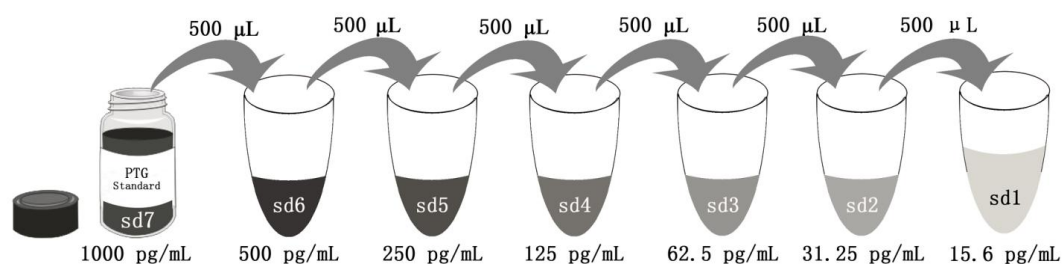
**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 4B1 is for standard, serum, plasma and cell culture supernatants.

Sample Diluent PT 4 is for standard, urine and saliva.

Detection Diluent is for Detection antibody.

\*Add 2 mL Sample Diluent PT 4B1 or PT 4 in 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 450nm 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.

## safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.

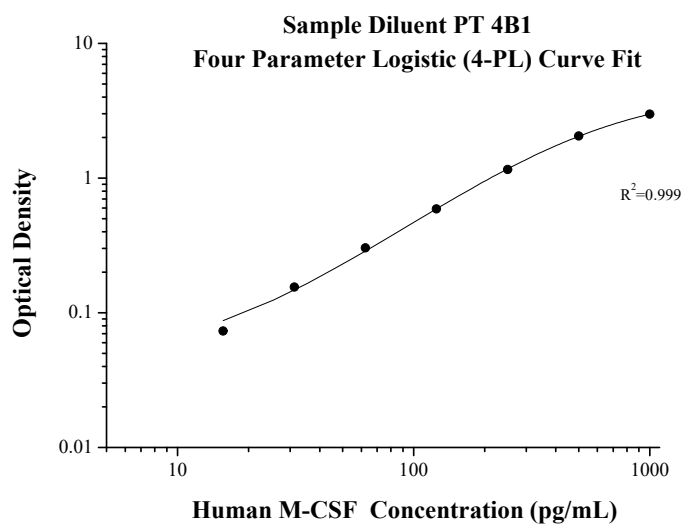
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## assay procedure summary

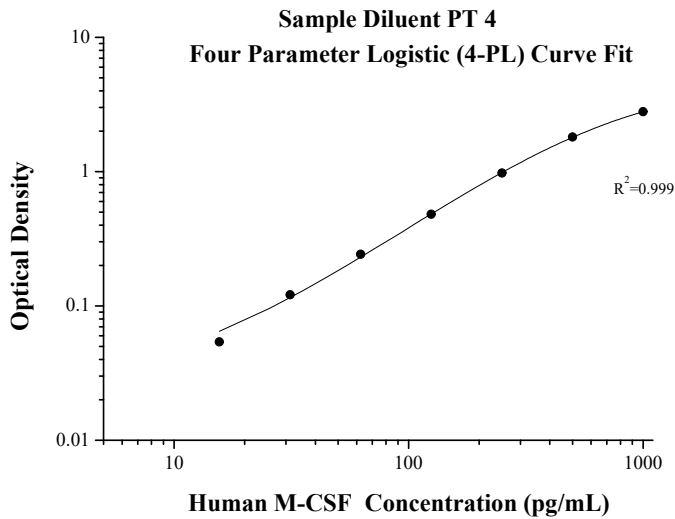
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	<b>120 min</b>	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 µL	<b>40 min</b>	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark 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.				

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



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

## 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)	207.3	433.8	797.3	214.6	475.3	880.9
SD	5.5	8.8	51.7	6.9	22.1	48.0
CV%	2.7	2.0	6.5	3.2	4.7	5.5

## 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

## 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) ( $1 \times 10^6$  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 µg/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 µg/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.

## linearity

To assess the linearity of the assay, serum and plasma samples were spiked with high concentrations of Human M-CSF and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. Cell culture supernatants 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	

## references

1. J W Pollard. et al. (1987) Nature. 330(6147): 484-6.
2. B R Witt. et al. (1997) Fertil Steril. 68(2): 259-64.
3. Anna Popova. et al. (2010) Immunobiology. 216(1-2):164-72.
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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.