PRODUCT INFORMATION & MANUAL

Human M-CSF Valukine™ ELISA

Catalog Number: VAL234

For the quantitative determination of natural and recombinant human Macrophage Colony Stimulating Factor (M-CSF) concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND

M-CSF, also known as CSF-1, is a four-α-helical-bundle cytokine that is the primary regulator of macrophage survival, proliferation, and differentiation (1-7). M-CSF is found as isoforms of various sizes. All isoforms contain the N-terminal 150 amino acid (aa) portion that is necessary and sufficient for interaction with the M-CSF receptor but may vary in activity and half-life (7-15). Full length human M-CSF transcripts encode a 522 aa type I transmembrane (TM) protein that forms a 140 kDa covalent dimer. Differential processing produces two proteolytically cleaved, secreted dimers. One is an N- and O-glycosylated 86 kDa dimer, while the other is modified by both glycosylation and chondroitin-sulfate proteoglycan (PG) to generate a 200 kDa subunit. Although PG-modified M-CSF can circulate, it may be immobilized by attachment to type V collagen (11). Shorter transcripts encode M-CSF that lacks cleavage and PG sites and produces an N-glycosylated 68 kDa TM dimer and a slowly produced 44 kDa secreted dimer (12). The region of mature human M-CSF that is common to all forms shares 88%, 86%, 81%, and 74% aa sequence identity with corresponding regions of canine, bovine, mouse, and rat M-CSF, respectively (1, 2). Human M-CSF is active in the mouse, but mouse M-CSF is reported to be species-specific. Sources of M-CSF include fibroblasts, activated macrophages, endometrial secretory epithelium, bone marrow stromal cells, vitamin D-stimulated osteoblasts, and activated endothelial cells (3-8, 16).

The M-CSF receptor (M-CSF R, also called *c-fms*) transduces the pleotropic effects of M-CSF and mediates its endocytosis. Engagement of M-CSF dimers by M-CSF R induces receptor dimerization, followed by phosphorylation at multiple sites (4, 14, 17, 18). M-CSF R is expressed on monocytes and tissue macrophages, and treatment with M-CSF promotes differentiation of macrophages, kidney mesangial cells, liver Kupffer cells, brain microglial cells, bone osteoclasts, fetal trophoblast cells, skin Langerhans cells, intestinal Paneth cells, and blood and lymph node plasmacytoid dendritic cells (3-5, 8, 19). M-CSF R is also expressed on osteoblasts where it downregulates RANKL production, thus allowing M-CSF to limit osteoclast production (20). IL-34 can also engage the M-CSF R, but downstream effects differ (21).

M-CSF is essential for macrophage-related functions such as bone resorption, vascular development, and innate immunity. M-CSF-deficient (op/op) mice are deficient in macrophages and are osteopetrotic due to insufficient differentiation of bone-resorbing osteoclasts (3, 6-8, 20, 22). They also show failure of teeth eruption, infertility, and defects in development of nervous, vascular, and lymphatic systems (4,

7, 16, 22). M-CSF regulates the release of cytokines and other inflammatory modulators from macrophages, and stimulates chemotaxis and pinocytosis (4, 5, 7). Circulating M-CSF increases during pregnancy and supports implantation and growth of the decidua and placenta (3, 7, 16). M-CSF expression can also be increased during infection or in inflammatory disorders such as inflammatory bowel disease (3, 5, 6). Both M-CSF and its receptor can be expressed by a number of cancers, allowing M-CSF to act as an autocrine growth factor for cancer cells. Macrophages can also be recruited to tumor tissues, supplying M-CSF as a paracrine growth factor (23). On the other hand, M-CSF can have anti-cancer effects by priming and enhancing macrophage killing of tumor cells and microorganisms (3). It is thought that expression of M-CSF R on cancer cells facilitates metastasis to the bone by chemotaxis toward osteoblastproduced M-CSF and by promoting osteolysis (3, 24).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human M-CSF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human M-CSF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human M-CSF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human M-CSF bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernates, human serum, human plasma, human saliva and human urine.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent RD5-18/RD6P and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in forty separate assays to assess inter-assay precision.

CELL CULTURE SUPERNATE/HUMAN SALIVA/HUMAN URINE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	248	751	1503	280	827	1620
Standard Deviation	19.4	16.1	49.0	30.2	76.0	87.7
CV%	7.8	2.1	3.3	10.8	9.2	5.4

HUMAN SERUM/PLASMA ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	357	951	1767	343	1019	1956
Standard Deviation	17.4	24.9	49.4	43.4	84.1	88.8
CV%	4.9	2.6	2.8	12.7	8.3	4.5

B. RECOVERY

The recovery of human M-CSF spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	99	90-107
Human Serum (n=4)	101	96-106
Human EDTA plasma (n=4)	100	93-106
Human Heparin plasma (n=4)	99	93-106

C. SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of M-CSF ranged from 1.74-47.3 pg/mL. The mean MDD was 11.2 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified 223 amino acid CHO cell-expressed recombinant human M-CSF produced at R&D Systems. The NIBSC/WHO 1st International recombinant Human M-CSF Standard 89/512 was evaluated in this kit. The dose response curve of the NIBSC standard 89/512 parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human M-CSF kit to approximate NIBSC International Units, use the equation below:

NIBSC (89/512) approximate value (IU/mL) = 0.042 × Valukine Human M-CSF value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human M-CSF and diluted with Calibrator Diluent RD5-18/RD6P to produce samples with values within the dynamic range of the assay.

Dilution		**Cell culture supernates (n=4)**	**Human Serum (n=4)**	**Human EDTA plasma (n=4)**	**Human Heparin plasma (n=4)**	**Human Saliva (n=4)**	**Human Urine (n=4)**
1:2	Average % of Expected	104	94	94	97	97	96
	Range (%)	97-109	90-98	91-99	90-102	92-107	92-103
1:4	Average % of Expected	103	97	101	102	93	94
	Range (%)	93-113	93-99	92-106	92-109	84-105	90-103

1:8	Average % of Expected	105	101	105	102	96	92
	Range (%)	91-115	98-108	100-112	95-109	88-103	84-106
1:16	Average % of Expected	101	104	106	98	88	98
	Range (%)	85-109	99-111	100-119	92-104	83-93	82-114

F. SAMPLE VALUES

Human Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human M-CSF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human Serum (n=36)	296	180-474	80.7
Human EDTA plasma (n=36)	207	108-374	65.2
Human Heparin plasma (n=36)	266	134-434	79.0
Human Saliva (n=7)	1312	312-2994	1100
Human Urine (n=14)	1413	263-4466	1336

Cell Culture Supernates:

Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL of PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human M-CSF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated cells	ND	156
Stimulated cells	205	933

ND=Non-detectable

A549 human lung carcinoma cells were cultured in MEM and supplemented with 5% fetal bovine serum until confluent. An aliquot of the cell culture supernate was removed, assayed for human M-CSF, and measured 5304 pg/mL.

U2OS human osteosarcoma cells were cultured in McCoy's 5a and supplemented with

15% fetal bovine serum until confluent. An aliquot of the cell culture supernate was removed, assayed for human M-CSF, and measured 156 pg/mL.

MG-63 human osteosarcoma cells were cultured in MEM supplemented with 10% fetal bovine serum and NEAA until confluent. An aliquot of the cell culture supernate was removed, assayed for human M-CSF, and measured 12,292 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human M-CSF.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-18/RD6P and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human M-CSF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:		**Recombinant mouse:**	**Recombinant rat:**
β-ECGF	M-CSF R	FGF-8b	GM-CSF
EGF	MSP	FGF-8c	β-NGF
FGF-4	β-NGF	Flt-3/Flk-2 Ligand	PDGF-AA
FGF-5	NRG1-α/HRG1-α	G-CSF	PDGF-AB
FGF-6	PD-ECGF	GM-CSF	PDGF-BB
FGF-9	PDGF-AA	M-CSF R	
FGF-10	PDGF-AB	$VEGF_{120}$	
FGF-18	PDGF-BB	$VEGF_{164}$	
FGF acidic	PDGF-CC	PDGF-CC	
FGF basic	PDGF-DD	PDGF Rα	

Flt-3/Flk-2 Ligand	PDGF Rα	PDGF Rβ	
Flt-4	PDGF Rβ	PlGF-2	
G-CSF	PlGF		
GM-CSF	$VEGF_{121}$		
HB-EGF	$VEGF_{165}$		
HGF	VEGF/PlGF		
IGF-I	VEGF-D		
IGF-II			
KGF/FGF-7			

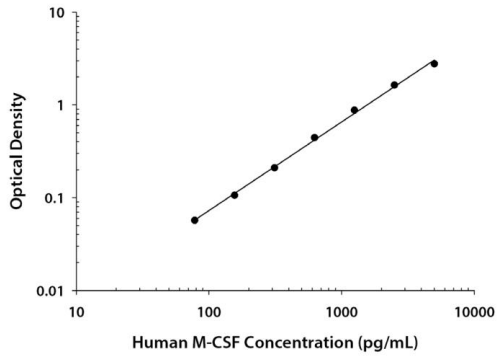
Recombinant mouse M-CSF cross-reacts approximately 0.18% in this assay.

IV. EXPERIMENT

EXAMPLE STANDARD

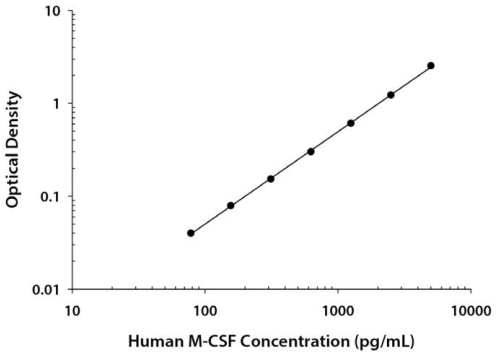
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.017 0.017	0.017	—
78.1	0.071 0.076	0.074	0.057
156	0.115 0.131	0.123	0.106
313	0.216 0.237	0.227	0.210
625	0.447 0.475	0.461	0.444
1250	0.868 0.917	0.893	0.876
2500	1.606 1.689	1.648	1.631
5000	2.610 2.958	2.784	2.767

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.037	0.037	—
78.1	0.071 0.074	0.073	0.036
156	0.106 0.115	0.111	0.074
313	0.177 0.187	0.182	0.146
625	0.341 0.356	0.349	0.312
1250	0.658 0.666	0.662	0.626
2500	1.279 1.348	1.314	1.277
5000	2.467 2.535	2.501	2.465

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human M-CSF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human M-CSF	1 plate
Human M-CSF Conjugate	Solution of antibody against human M-CSF conjugated to horseradish peroxidase	1 vial
Human M-CSF Standard	Recombinant human M-CSF in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1-56	A buffered protein base with blue dye	1 vial
Calibrator Diluent RD5-18	A buffered protein base used to dilute standard and samples (*For cell culture supernate/human saliva/human urine samples*)	1 vial
Calibrator Diluent RD6P	Animal serum used to dilute standard and samples (*For human serum/plasma samples*)	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Sealers	Adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8°C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1-56</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD5-18</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD6P</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
- Human M-CSF is detectable in human saliva. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-18.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6P.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6P.

Note: *Citrate plasma is not recommended for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 × g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-18.

Note: *Saliva values are decreased when a Salivette or other collection device is used. When stored at 2-8 °C, saliva sample values increase over time.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-18.

B. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of human M-CSF are found in human saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

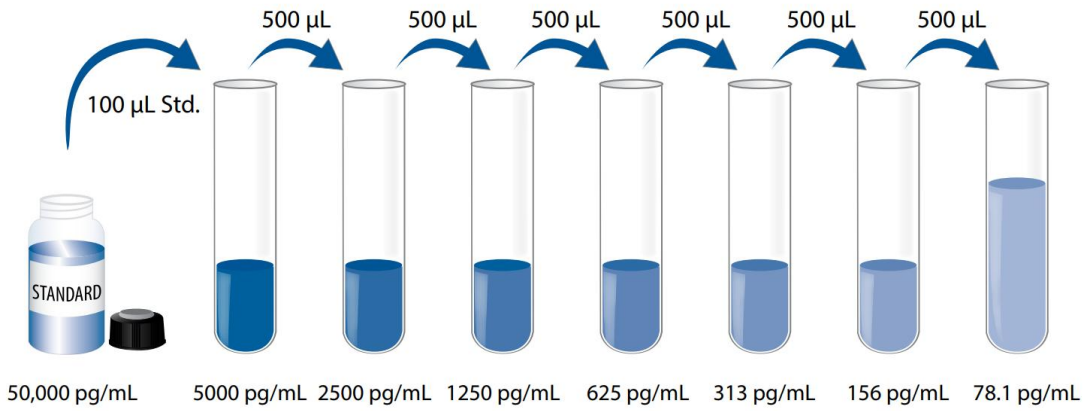
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Human M-CSF Standard - Refer to the vial label for the reconstitution volume*.

Reconstitute the Human M-CSF Standard with deionized or distilled water. This reconstitution produces a stock solution of 50000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Pipette 900 µL of Calibrator Diluent RD5-18 (*for cell culture supernate/human saliva/human urine samples*) or Calibrator Diluent RD6P (*for human serum/plasma samples*) into the 5000 pg/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



C. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

VII.ASSAY PROCEDURE

Bring all other reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

Note: *High concentrations of human M-CSF are found in human saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-56 to each well.
4. Add 100 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human M-CSF Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature on the benchtop. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

11. CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human M-CSF concentrations versus the log of the O.D. on a linear scale and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

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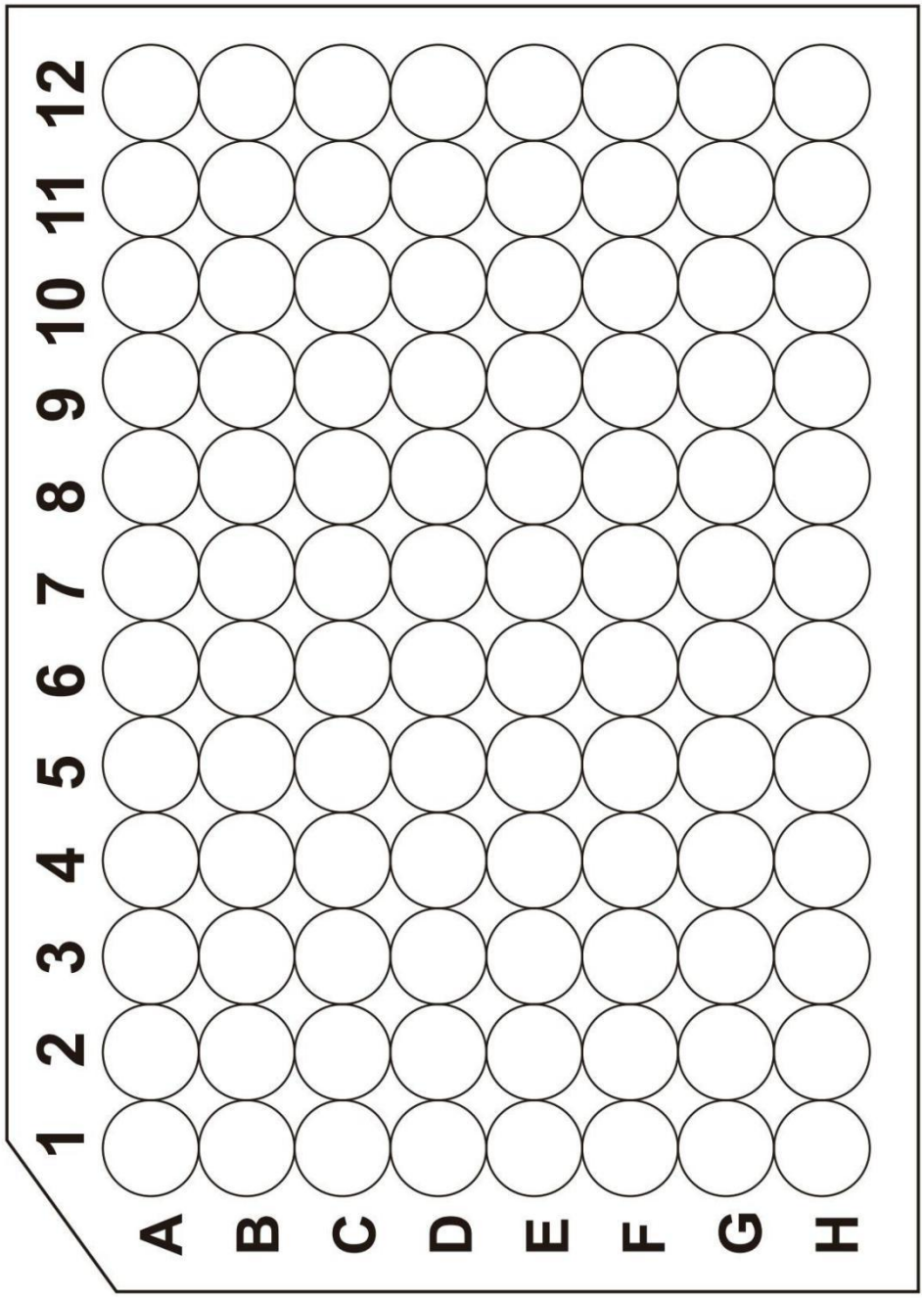
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 M-CSF Valukine™ ELISA 试剂盒

目录号：VAL234

适用于定量检测天然和重组人巨噬细胞集落刺激因子（M-CSF）的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

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版本号 202411.1

目录

I. 背景

M-CSF 又称 CSF-1，是一种四α螺旋束细胞因子，是巨噬细胞存活、增殖和分化的主要调节因子（1-7）。M-CSF 有不同大小的异构体。所有异构体都含有 N 端 150 个氨基酸（amino acid，aa）部分，该部分是与 M-CSF 受体相互作用的必要和充分条件，但其活性和半衰期可能有所不同（7-15）。全长类 M-CSF 转录本编码 522 aa 的 I 型跨膜（transmembrane，TM）蛋白，可形成 140 kDa 的共价二聚体。不同的加工过程会产生两种蛋白水解裂解的分泌型二聚体。其中一个是 N 和 O-糖基化的 86 kDa 二聚体，而另一个则经过糖基化和硫酸软骨素蛋白多糖（proteoglycan，PG）修饰，生成一个 200 kDa 的亚基。虽然 PG 修饰的 M-CSF 可以循环，但它可能会因附着在 V 型胶原上而被固定（11）。较短的转录本编码的 M-CSF 缺乏裂解位点和 PG 位点，可产生 N 糖基化的 68 kDa TM 二聚体和分泌缓慢的 44 kDa 二聚体（12）。成熟的人 M-CSF 与犬、牛、小鼠和大鼠 M-CSF 的相应区域分别有 88%、86%、81%和 74%的 aa 序列相同性（1, 2）。人的 M-CSF 在小鼠体内也有活性，但据报道小鼠的 M-CSF 具有物种特异性。M-CSF 的来源包括成纤维细胞、活化的巨噬细胞、子宫内膜分泌上皮细胞、骨髓基质细胞、维生素 D 刺激的成骨细胞和活化的内皮细胞（3-8, 16）。

M-CSF 受体（M-CSF R，又称 *c-fms*）可转导 M-CSF 的多向性效应，并介导其内吞作用。M-CSF R 与 M-CSF 二聚体接触后会诱导受体二聚化，随后在多个位点发生磷酸化（4, 14, 17, 18）。M-CSF R 在单核细胞和组织巨噬细胞上表达，用 M-CSF 处理可促进巨噬细胞、肾间质细胞、肝 Kupffer 细胞、脑小胶质细胞、骨破骨细胞、胎儿滋养细胞、皮肤朗格汉斯细胞、肠 Paneth 细胞以及血液和淋巴结浆细胞树突状细胞的分化（3-5, 8, 19）。M-CSF R 也在成骨细胞上表达，它能下调 RANKL 的生成，从而使 M-CSF 限制破骨细胞的生成（20）。IL-34 也能参与 M-CSF R，但下游效应不同（21）。

M-CSF 对巨噬细胞的相关功能至关重要，如骨吸收、血管发育和先天免疫。缺乏 M-CSF 的小鼠（op/op）缺乏巨噬细胞，并且由于骨吸收破骨细胞分化不足而导致骨质疏松（3, 6-8, 20, 22）。他们还表现出牙齿萌出失败、不育以及神经、血管和淋巴系统发育缺陷（4, 7, 16, 22）。M-CSF 可调节巨噬细胞释放细胞因子和其他炎症调节剂，并刺激趋化和胞饮作用（4, 5, 7）。怀孕期间，循环中的 M-CSF 会增加，并支持蜕膜和胎盘的植入和生长（3, 7, 16）。在感染或炎症性疾病（如炎症性肠病）时，M-CSF 的表达也会增加（3, 5, 6）。许多癌症都能表达 M-CSF 及其受体，从而使 M-CSF 成为癌细胞的自分泌生长因子。巨噬细胞也可被招募到肿瘤组织，提供 M-CSF 作为旁分泌生长因子（23）。另一方面，M-CSF 可通过启动和增强巨噬细胞对肿瘤细胞和微生物的杀伤力来发挥抗癌作用（3）。据认为，癌细胞表达的 M-CSF R 可通过对成骨细胞产生的 M-CSF 的趋化作用和促进溶骨作用，促进向骨骼的转移（3, 24）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人M-CSF抗体包被于微孔板上，样品和标准品中的人M-CSF会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人M-CSF检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断**。
- 该试剂盒适用于细胞培养上清液、人血清、人血浆、人唾液和人尿液。
- 请在试剂盒有效期内使用。
- 不同试剂盒及不同批号试剂盒的组分不能混用。
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液RD5-18/RD6P稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测40次，以确定板间精确度。

细胞培养上清液/人唾液/人尿液检测

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（pg/mL）	248	751	1503	280	827	1620
标准差	19.4	16.1	49.0	30.2	76.0	87.7
CV%	7.8	2.1	3.3	10.8	9.2	5.4

人血清/血浆检测

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（pg/mL）	357	951	1767	343	1019	1956
标准差	17.4	24.9	49.4	43.4	84.1	88.8
CV%	4.9	2.6	2.8	12.7	8.3	4.5

B. 回收率

不同类型样本中掺入检测范围内不同水平的人M-CSF，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基（n=4）	99	90-107
人血清（n=4）	101	96-106
人 EDTA 血浆（n=4）	100	93-106
人肝素血浆（n=4）	99	93-106

C. 灵敏度

评估 80 次检测，人 M-CSF 的最小可检测剂量（MDD）范围为 1.74-47.3 pg/mL，平均 MDD 为 11.2 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

该免疫测定法以R&D Systems生产的高纯度的CHO细胞表达的重组人M-CSF（223个氨基酸）校正。该试剂盒中使用NIBSC/WHO第一代重组人M-CSF国际标准品89/512来评估。NIBSC标准品89/512的剂量反应曲线与Valukine标准曲线平行。将Valukine人M-CSF试剂盒获得的样品值转换为近似的NIBSC国际单位值，请使用以下公式：

NIBSC（89/512）近似值（IU/mL）= 0.042 × Valukine人M-CSF值（pg/mL）

E. 线性

不同的样本中含有或掺入高浓度的人M-CSF，然后用标准品稀释液RD5-18/RD6P将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养上清液（n=4）	人血清（n=4）	人EDTA血浆（n=4）	人肝素血浆（n=4）	人唾液（n=4）	人尿液（n=4）
1:2	平均值/期待值（%）	104	94	94	97	97	96
	范围（%）	97-109	90-98	91-99	90-102	92-107	92-103
1:4	平均值/期待值（%）	103	97	101	102	93	94
	范围（%）	93-113	93-99	92-106	92-109	84-105	90-103
1:8	平均值/期待值（%）	105	101	105	102	96	92
	范围（%）	91-115	98-108	100-112	95-109	88-103	84-106
1:16	平均值/期待值（%）	101	104	106	98	88	98
	范围（%）	85-109	99-111	100-119	92-104	83-93	82-114

F. 样本预值

人血清/血浆/唾液/尿液 - 在本试验中评估了来自表面健康志愿者的样本中是否存在人 M-CSF。本研究中使用的供体没有病史。

样本类型	平均值（pg/mL）	范围（pg/mL）	标准差（pg/mL）
人血清（n=36）	296	180-474	80.7
人EDTA血浆（n=36）	207	108-374	65.2
人肝素血浆（n=36）	266	134-434	79.0
人唾液（n=7）	1312	312-2994	1100
人尿液（n=14）	1413	263-4466	1336

细胞培养上清：

人外周血白细胞在含有5%胎牛血清、5 μM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL链霉素硫酸盐的DMEM培养基中培养。细胞在不刺激或用10 μg/mL PHA刺激1天和5天。取细胞培养上清液测定人M-CSF的浓度。

条件	1 天（pg/mL）	5 天（pg/mL）
未刺激	ND	156
刺激	205	933

ND=未检出

将A549人肺癌细胞在添加5%胎牛血清的MEM中培养，直至汇合。取细胞培养上清液测定人M-CSF的浓度，结果为5304 pg/mL。

将U2OS人骨肉瘤细胞在添加15%胎牛血清的McCoy's 5a中培养，直至汇合。取细胞培养上清液测定人M-CSF的浓度，结果为156 pg/mL。

将MG-63人骨肉瘤细胞在添加10%胎牛血清和NEAA的MEM中培养，直至汇合。取细胞培养上清液测定人M-CSF的浓度，结果为12,292 pg/mL。

G. 特异性

检测方法识别天然和重组人M-CSF。

以下列出的因子在标准品稀释液RD5-18/RD6P中以50 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人M-CSF对照品中以50 ng/mL的浓度制

备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human:		**Recombinant mouse:**	**Recombinant rat:**
β-ECGF	M-CSF R	FGF-8b	GM-CSF
EGF	MSP	FGF-8c	β-NGF
FGF-4	β-NGF	Flt-3/Flk-2 Ligand	PDGF-AA
FGF-5	NRG1-α/HRG1-α	G-CSF	PDGF-AB
FGF-6	PD-ECGF	GM-CSF	PDGF-BB
FGF-9	PDGF-AA	M-CSF R	
FGF-10	PDGF-AB	$VEGF_{120}$	
FGF-18	PDGF-BB	$VEGF_{164}$	
FGF acidic	PDGF-CC	PDGF-CC	
FGF basic	PDGF-DD	PDGF Rα	
Flt-3/Flk-2 Ligand	PDGF Rα	PDGF Rβ	
Flt-4	PDGF Rβ	PlGF-2	
G-CSF	PlGF		
GM-CSF	$VEGF_{121}$		
HB-EGF	$VEGF_{165}$		
HGF	VEGF/PlGF		
IGF-I	VEGF-D		
IGF-II			
KGF/FGF-7			

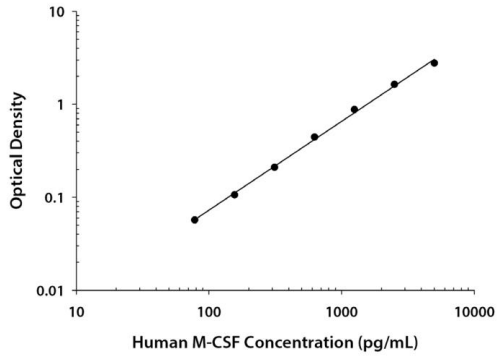
本试验中，重组鼠M-CSF交叉反应率约为0.18%。

IV. 实验

标准曲线实例

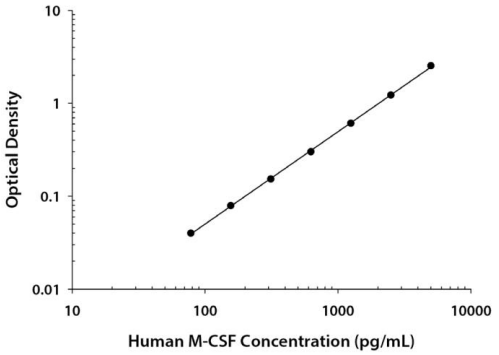
这些标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.017 0.017	0.017	—
78.1	0.071 0.076	0.074	0.057
156	0.115 0.131	0.123	0.106
313	0.216 0.237	0.227	0.210
625	0.447 0.475	0.461	0.444
1250	0.868 0.917	0.893	0.876
2500	1.606 1.689	1.648	1.631
5000	2.610 2.958	2.784	2.767

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.037	0.037	—
78.1	0.071 0.074	0.073	0.036
156	0.106 0.115	0.111	0.074
313	0.177 0.187	0.182	0.146
625	0.341 0.356	0.349	0.312
1250	0.658 0.666	0.662	0.626
2500	1.279 1.348	1.314	1.277
5000	2.467 2.535	2.501	2.465

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human M-CSF Microplate	包被抗人M-CSF抗体的96孔聚苯乙烯板，8孔 × 12条	1块板
Human M-CSF Conjugate	酶标检测抗人M-CSF抗体	1瓶
Human M-CSF Standard	重组人M-CSF标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-56	蓝色检测液	1瓶
Calibrator Diluent RD5-18	标准品稀释液，用于稀释标准品和样品 （*用于细胞培养上清液/人唾液/人尿液样本*）	1瓶
Calibrator Diluent RD6P	标准品稀释液，用于稀释标准品和样品 （*用于人血清/人血浆样本*）	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

未开封试剂盒	2-8°C储存；请在试剂盒有效期内使用	
已打开，稀释或重溶的试剂	洗涤液（1×）	2-8°C储存，最多30天*
	终止液	
	酶标检测抗体	
	TMB底物溶液	
	标准品	2-8°C储存，最多30天*
	检测液RD1-56	2-8°C储存，最多30天*
	标准品稀释液RD5-18	2-8°C 储存，最多 30 天*
	标准品稀释液RD6P	2-8°C 储存，最多 30 天*
	包被的微孔板条	将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8°C储存，最多30天*

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 500 mL量筒
- 用于稀释标准品和样品的管子

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。
- 人M-CSF可在人唾液中检测到。进行检测时，请采取预防措施，避免试剂盒试剂的污染。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液RD5-18稀释。

血清 - 使用血清分离管(SST)，让样本在室温下凝固30分钟，然后在1000 × g的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融。样本可能需要用标准品稀释液RD6P稀释。

血浆 - 使用肝素或EDTA作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD6P稀释。

注： *柠檬酸盐血浆不建议在本检测中使用。*

唾液 - 将唾液收集到管中，以10000 × g离心5分钟。收集水层，立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD5-18稀释。

注： *使用唾液收集器（如Salivette）等装置时，唾液样本值会降低。在2-8°C保存时，唾液样本值会随时间增加。*

尿液 - 无菌采集当日首次尿液（中段尿），直接排入无菌容器中。离心去除颗粒物质，立即检测或分装后储存于≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD5-18进行稀释。

B. 检测前准备工作

使用前请将所有试剂放置于室温。

注： *人M-CSF在人体唾液中浓度较高。建议使用口罩和手套以防止试剂盒试剂受污染。*

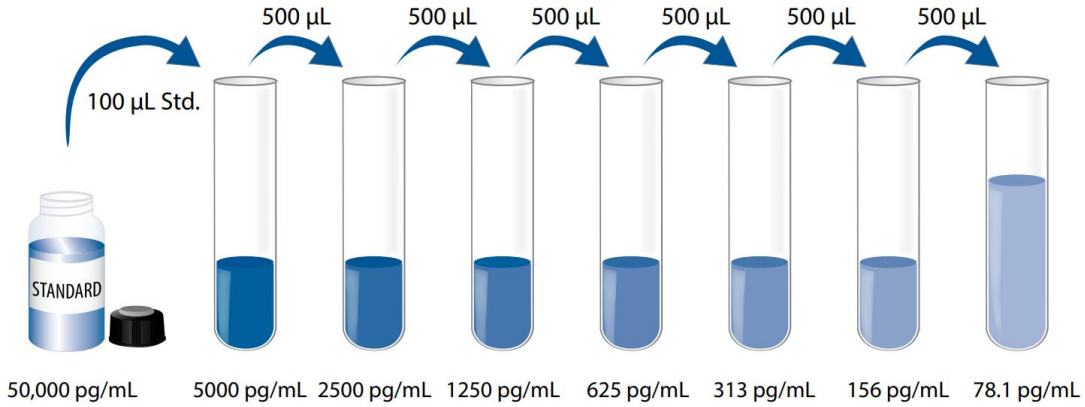
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

人M-CSF标准品：复溶体积请参考瓶身标签*，用去离子水或蒸馏水复溶人M-CSF标准品，得到浓度为50000 pg/mL标准品储备母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

用移液管将 900 µL 标准品稀释液 RD5-18（*用于细胞培养上清液/人唾液/人尿液样本*）或标准品稀释液 RD6P（*用于人血清/人血浆样本*）移入 5000 pg/mL 的管中，其余管中加入 500 µL 标准品稀释液。使用标准品储备母液稀释（如下图所示）。在每次转移之

前，将每个管子彻底混合。5000 pg/mL 作为标准曲线的最高点。相应的标准品稀释液作为标准曲线的零点（0 pg/mL）。



C. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫。
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽。
- 建议15分钟内完成一块板的上样。
- 每次孵育时，正确使用封板膜可保证结果的准确性。
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色。
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合。

VII.操作步骤

使用前，将所有其他试剂和样品放置于室温。建议对所有标准品和样品进行复孔检测。

注：*人M-CSF在人体唾液中浓度较高。建议使用口罩和手套以防止试剂盒试剂受污染。*

1. 按照上一节的说明，准备好所有需要的试剂和标准品。
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口。
3. 向每个孔中加入100 µL的检测液RD1-56。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，**室温孵育2小时**。说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置。
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体。
6. 在每个微孔内加入200 µL人M-CSF酶标检测抗体。用新的封板膜封住反应孔，**室温孵育2小时**。
7. 重复第5步洗板操作。
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**。
9. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀。
10. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确。
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作log/log曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来建立标准曲线，并通过log/log图上的点绘制最佳拟合曲线。数据可以通过绘制人M-CSF浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置