PRODUCT INFORMATION & MANUAL

Human MMP-9 Valukine™ ELISA

VAL113

For the quantitative determination of human active (82 kDa) and Pro- (92 kDa) Matrix Metalloproteinase 9 (MMP-9) 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 202609.4

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I. BACKGROUND

Matrix metalloproteinases (MMPs), also called matrixins, constitute a family of zinc and calcium dependent endopeptidases that function in the breakdown of the extracellular matrix (ECM) and in the processing of a variety of molecules in different subcellular environments. They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction, and tissue remodeling (1, 2). They also participate in inflammatory and autoimmune disorders such as arthritis, cancer, and cardiovascular disease (3-5). While the amounts of newly synthesized MMPs are regulated mainly at the levels of transcription, the proteolytic activities of existing MMPs are controlled through both the activation of proenzymes or zymogens and the inhibition of active enzymes by endogenous inhibitors, α2-Macroglobulin, and tissue inhibitors of metalloproteinases (TIMPs) (6).

MMP-9 (also referred to as gelatinase B, 92 kDa type IV collagenase, 92 kDa gelatinase, and type V collagenase) is secreted as a glycosylated proenzyme (6-8). Activation of the proenzyme involves proteolytic removal of the N-terminal pro region, resulting in the 82 kDa active enzyme (9, 10). Active human MMP-9 shares 72% and 74% amino acid sequence identity with mouse and rat MMP-9, respectively. In addition to the zinc-binding site, the catalytic domain also contains three contiguous fibronectin type II homology units responsible for binding gelatin (11). A proline-rich hinge region links the catalytic domain to the C-terminal hemopexin-like domain. In vitro treatment of the proenzyme with 4-aminophenylmercuric acetate (APMA) produces not only the active enzyme but also a C-terminal truncated form with activity comparable to that of the active form (12). MMP-9 degrades components of the ECM with high specific activity for denatured collagens (gelatin). It can cleave native collagens of type III, IV, V, and XI, as well as Elastin, Nidogen-1, and Vitronectin (2, 3). MMP-9 can also cleave a variety of chemokines and growth factors (e.g. IL-1β, CXCL8/IL-8, CXCL7, CXCL4, CXCL1, Latent TGF-β, membrane bound TNF-α, VEGF, and FGF basic), Amyloid β peptide, Substance P, and Myelin Basic Protein (3, 13-15). This action can increase or decrease the biological activity of soluble factors and can also liberate them from association with the ECM (16, 17). MMP-9 can also trigger signaling through various transmembrane proteins or inhibit signaling by inducing their shedding from the cell surface (e.g. CD44, E-Cadherin, Integrins, ICAM-1, and IL-2 Rα) (3, 18-20).

MMP-9 is produced by a variety of normal and transformed cells including neutrophils, monocytes, macrophages, astrocytes, fibroblasts, osteoclasts, chondrocytes, keratinocytes, endothelial and epithelial cells. It exerts physiological and pathological angiogenitic and remodeling effects on the vasculature (21-25). Activated neutrophils release proMMP-9 which is free of TIMP-1, allowing the liberation of pro-angiogenic FGF-2 from the ECM (17). MMP-9 in complex with TIMP-1 does not induce FGF-2 release (17). Neutrophil-derived MMP-9 exacerbates the inflammatory response, in part by generating collagen-derived peptides that induce the release of additional neutrophil MMP-9 (26). MMP-9 also pays a role in bone formation and remodeling (1, 21, 27), methamphetamine induced behavioral sensitization and reward (28), the regulation of neuronal synapse remodeling (29), trophoblast invasion during implantation (30), and the inactivation of Serpin α1-Proteinase Inhibitor (31). The shedding of adhesion proteins by MMP-9 has a direct effect on tumor cell invasiveness (18-20). Circulating levels of MMP-9 are increased in many inflammatory disorders including intraluminal thrombus formation (32), atherosclerosis (33), Crohn's disease (34), hepatitis C virus infection (35), colorectal cancer (36), and Duchenne muscular dystrophy (37). The ratio of MMP-9 to TIMP-1 is also increased in multiple sclerosis serum (38) and cystic fibrosis sputum (39), but it is decreased in the serum during cytomegalovirus infection (40). Levels of free MMP-9 and complexes of MMP-9 with Lipocalin-2/NGAL are elevated in the urine of ovarian cancer and uterine tract infection patients, respectively (41, 42).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human MMP-9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human MMP-9 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for MMP-9 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human MMP-9 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 supernate, human serum, human platelet-poor 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 RD5-10 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.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (ng/mL)	0.833	2.04	11.0	0.972	2.35	12.2
Standard Deviation	0.017	0.039	0.316	0.077	0.184	0.845
CV%	2.0	1.9	2.9	7.9	7.8	6.9

B. RECOVERY

The recovery of human MMP-9 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media* (n=5)	97	85-104
Serum* (n=5)	95	91-99
Platelet-poor heparin plasma* (n=5)	96	89-108
Saliva* (n=4)	90	74-114
Urine (n=5)	91	82-99

*Samples were diluted prior to assay as directed in the Sample Preparation section.

C. SENSITIVITY

The minimum detectable dose (MDD) of MMP-9 is typically less than 0.156 ng/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 highly purified *CHO cell*-expressed recombinant human Pro-MMP-9 produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of MMP-9 and diluted with Calibrator Diluent RD5-10 to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=5)	**Serum* (n=5)**	**Platelet-poor Heparin plasma* (n=5)**	**Saliva* (n=4)**	**Urine (n=4)**
1:2	Average % of Expected	104	103	102	105	101
	Range (%)	101-107	99-107	98-107	103-106	96-109
1:4	Average % of Expected	104	103	102	108	103
	Range (%)	101-106	99-108	99-106	103-109	95-110
1:8	Average % of Expected	102	102	104	111	104
	Range (%)	97-110	96-108	101-110	108-114	94-112
1:16	Average % of Expected	102	102	103	110	97
	Range (%)	96-110	95-108	95-110	109-112	90-109

*Samples were diluted prior to assay as directed in the Sample Preparation section.

F. SAMPLE VALUES

Serum/Platelet-poor Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human MMP-9 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Serum (n=37)	436	100	169-705
Platelet-poor heparin plasma (n=37)	32	100	13.2-105
Saliva (n=4)	201	100	102-543
Urine (n=53)	3.65	45	ND-33.6

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (5×10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 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 PHA. Aliquots of the cell culture supernate was removed on day 1 and 5 and assayed for human MMP-9.

Condition	Day 1 (ng/mL)	Day 3 (ng/mL)
Unstimulated	132	33.8
Stimulated	522	210

G. SPECIFICITY

This assay recognizes both natural and recombinant 92 kDa Pro-MMP-9 and 82 kDa active MMP-9. It does not measure the 65 kDa form.

The following factors were prepared at 100 or 200 ng/mL in Diluent 1x and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range rhMMP-9 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Human		Recombinant mouse
ADAM8	MMP-8	ADAM9
ADAM10	MMP-10	ADAM10
ADAM15	MMP-12	ADAM15
Lipocalin-2/NGAL	MMP-13	Lipocalin-2/NGAL
MMP-1	TACE (ADAM17)	MMP-2
MMP-2	TIMP-2	MMP-3
MMP-3	TIMP-3	MMP-9
MMP-7	TIMP-4	TIMP-1

No cross-reactivity was observed with recombinant human TIMP-1, but interference was observed at concentrations ≥ 6.25 ng/mL.

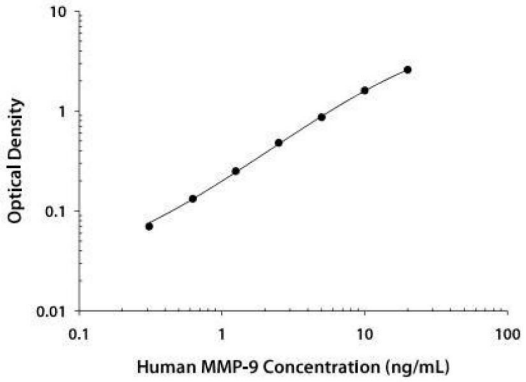
rhTIMP-1 Concentration (ng/mL)	Observed Value (ng/mL)
200	1.241
100	2.026
50	2.920
25	3.651
12.5	4.655
6.25	5.219
0	5.633

Interference was also observed with recombinant rat TIMP-1 at concentrations ≥ 200 ng/mL.

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.040 0.041	0.040	—
0.313	0.111 0.110	0.110	0.070
0.625	0.172 0.170	0.172	0.132
1.25	0.292 0.288	0.290	0.250
2.5	0.532 0.508	0.520	0.480
5	0.910 0.899	0.904	0.864
10	1.612 1.668	1.640	1.600
20	2.607 2.638	2.622	2.582

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
MMP-9 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human MMP-9	1 plate
MMP-9 Conjugate	An antibody against MMP-9 conjugated to horseradish peroxidase with preservatives	1 vial
Assay Diluent RD1-34	A buffered protein base with preservatives	1 vial
MMP-9 Standard	Recombinant human MMP-9 in a buffered protein base with preservatives; lyophilized	2 vials
Calibrator Diluent RD5-10	A buffered protein base with preservatives	3 vials
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.*	1 vial
TMB Substrate	TMB ELISA Substrate Solution	2 vials
Stop Solution	2 N sulfuric acid	1 vial
Plate Covers	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="5">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent RD1-34</td></tr>
<tr><td>Calibrator Diluent RD5-10</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Use a new standard for each assay. Discard after use.</td></tr>
<tr><td>Standard</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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- 500 mL graduated cylinder.
- Polypropylene tubes for dilution of standards and samples.

D. PRECAUTION

- MMP-9 is detectable in saliva. Use a face mask and gloves to protect kit reagents from contamination.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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 RD5-10.

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 RD5-10.

Platelet-Poor Plasma - Collect plasma on ice using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 × g for 10 minutes at 2-8°C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Note: *EDTA and citrate are not recommended anticoagulants for use in this assay due to their chelating properties.*

MMP-9 is released upon platelet activation. To measure circulating levels of MMP-9, platelet-free plasma should be used for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory and Standards Institute (CLSI), result in incomplete removal of platelets or platelet activation. This may cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

Saliva - Collect saliva using a collection device such as a Salivette™ or equivalent. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5-10.

Note: *Saliva collector cannot have any enzyme binding or filtering capabilities.*

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 RD5-10.

B. SAMPLE PREPARATION

Use polypropylene tubes.

Serum, cell culture supernate, and saliva samples require at least a 100-fold dilution prior to the assay. A suggested 100-fold dilution is 10 µL of Activated sample + 990 µL of Calibrator Diluent RD5-10. Optimal dilutions should be determined by the end user.

Platelet-poor plasma samples require at least a 40-fold dilution into Calibrator Diluent RD5-10. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

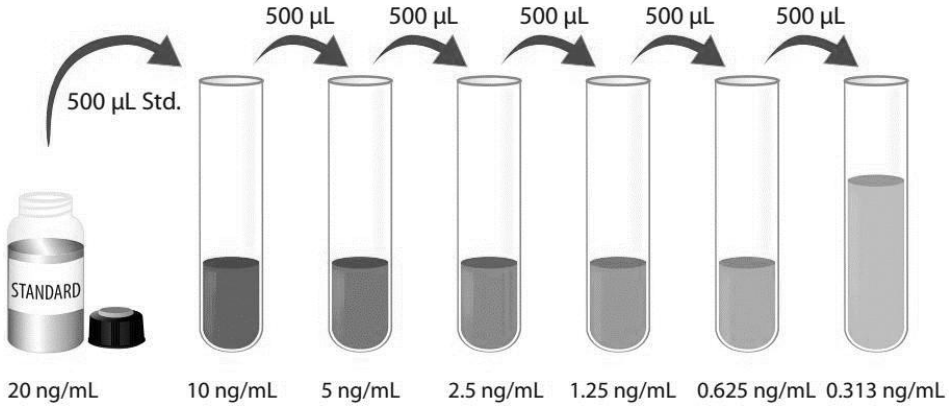
Note: *High concentrations of MMP-9 are found in saliva. Use a face mask and gloves to protect kit reagents from contamination.*

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 MMP-9 Standard- Refer to the vial label for reconstitution volume*. Reconstitute the Human MMP-9 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20 ng/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.

Use polypropylene tubes. Pipette 500 µL of the appropriate Calibrator Diluent RD5-10 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. The appropriate Calibrator Diluent RD5-10 serves as the zero standard (0 ng/mL).



D. 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 Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. 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 Substrate Solution.

VII. ASSAY PROCEDURE

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

Note: *High concentrations of MMP-9 are found in 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 plat frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-34 to each well.
4. Add 100 µL of Standard and sample per well. Cover with the adhesive strip provided. I**ncubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.** 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 Solution (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 MMP-9 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour 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. Protect from light**.
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, 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. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph. The data may be linearized by plotting the log of the MMP-9 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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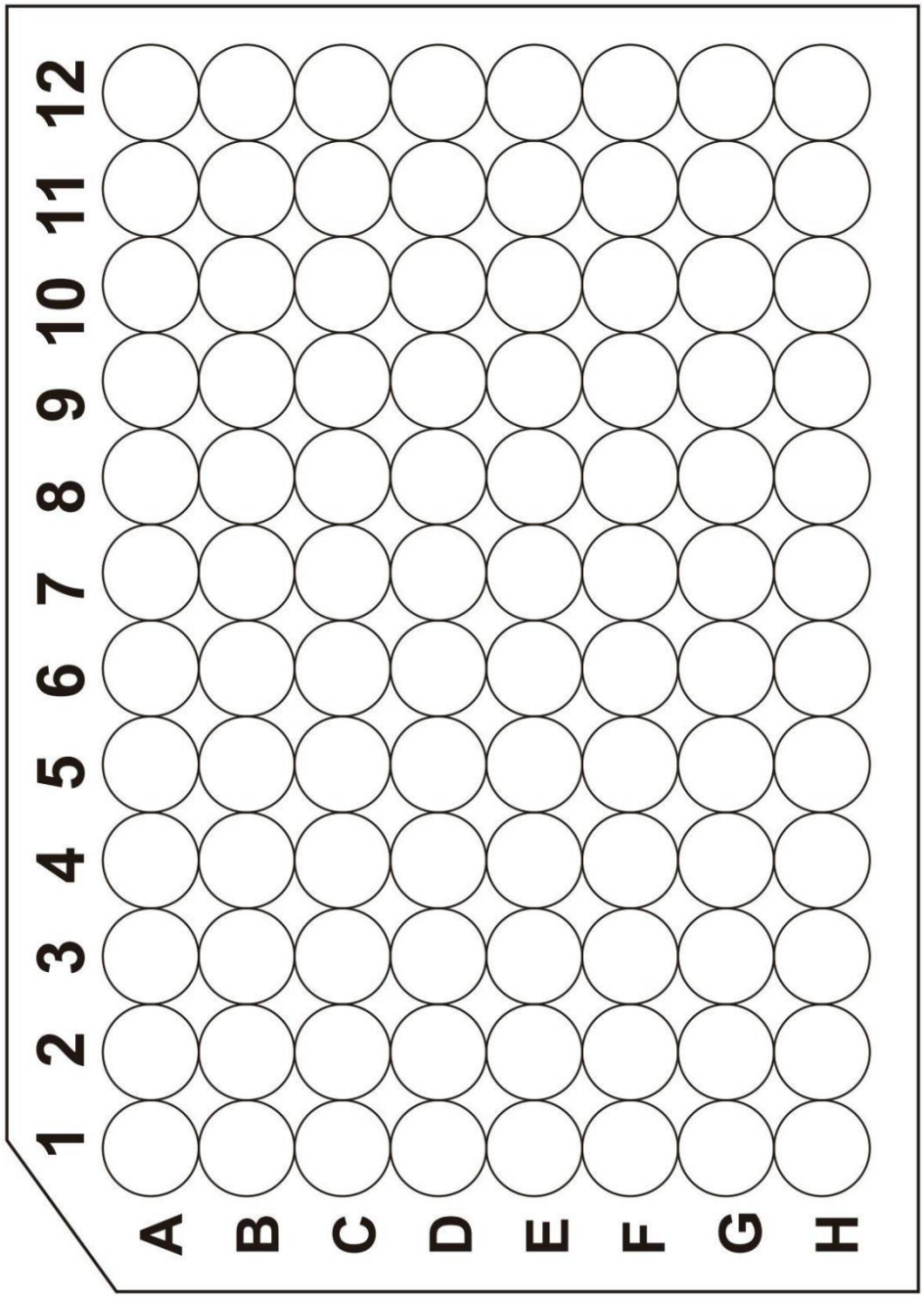
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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 MMP-9 Valukine™ ELISA 试剂盒

目录号：VAL113

适用于定量检测人活性型（82 kDa）及前体型（92 kDa）基质金属蛋白酶 9（MMP-9）的含量

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

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有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202609.4

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I. 背景

基质金属蛋白酶（MMPs）也被称为matrixins，是锌钙依赖性蛋白水解酶家族成员，可分解细胞外基质（ECM），在不同的亚细胞环境中，参与多种分子的加工过程。MMPs在众多生理学进程中发挥重要功能，例如胚胎发育、形态发生、繁殖以及组织重构（1, 2）。也参与炎症以及自身免疫疾病，例如关节炎、癌症和心血管疾病（3-5）。新合成的MMPs的量主要在转录水平被调节，已经存在的MMPs的蛋白水解活性，不仅受控于酶原的活性，也受控于内源性抑制剂对酶活性的抑制，例如α2-巨球蛋白，以及金属蛋白酶组织抑制剂（TIMPs）（6）。

MMP-9（也称明胶酶B、92 kDa IV型胶原酶、92 kDa明胶酶、V型胶原酶）作为糖基化酶原被分泌出来（6-8）。酶原的活化涉及到N-末端前体区域的蛋白水解去除，最终形成82 kDa的活性酶（9, 10）。活性人MMP-9与小鼠和大鼠的MMP-9分别有72%和 74%的氨基酸序列同源性。除了锌结合位点，催化结构域还包含三个连续的纤连蛋白II 型同源单位，可结合明胶（11）。富含脯氨酸的铰链区将催化结构域连接到C-末端血红素样结构域。用4-氨基苯汞乙酸盐（APMA）在体外处理酶原，不仅能产生活性酶，还能产生活性与其相当的 C-末端截短形式（12）。MMP-9可降解ECM中的成分，尤其对变性胶原（明胶）有非常高的特异性。MMP-9也可裂解III、IV、V、XI型胶原，以及弹性蛋白、巢蛋白-1和玻连蛋白（2, 3）。MMP-9还可裂解多种趋化因子和生长因子（例如IL-1β、CXCL8/IL-8、CXCL7、CXCL4、CXCL1、Latent TGF-β、膜结合TNF-α、VEGF和FGF basic），β-淀粉样蛋白、P物质、髓鞘碱性蛋白（3, 13-15）。此动作可提高或降低这些可溶性因子的生物活性，也可使它们从与ECM的联合中被释放出来（16, 17）。MMP-9也可通过各种各样的膜蛋白触发信号通路，或是诱导它们从细胞膜上脱落从而抑制信号通路（例如CD44，E-钙黏蛋白、整合素、ICAM-1和IL-2 Rα）（3, 18-20）。

MMP-9由多种正常细胞和转化细胞产生，包括中性粒细胞、单核细胞、巨噬细胞、星形胶质细胞、纤维原细胞、破骨细胞、软骨细胞、角化细胞、内皮细胞和上皮细胞。它影响生理性和病理性的血管生成和血管重构（21-25）。活化的中性粒细胞释放 MMP-9前体，不结合TIMP-1，允许促血管生成的FGF-2从ECM中释放出来（17）。MMP-9 和TIMP-1的复合物不能诱导FGF-2的释放（17）。中性粒细胞来源的MMP-9可加剧炎症反应，通过产生来自胶原多肽来诱导额外的中性粒细胞MMP-9的释放（26）。MMP-9在骨形成和重构（1, 21, 27）、甲基苯丙胺诱导行为敏化与应答（28）、神经元突触重构的调控（29）、着床过程中的滋养层入侵（30）、丝氨酸蛋白酶抑制剂α1的活化（31）

中也发挥重要作用。MMP-9介导的粘附分子的脱落对于肿瘤细胞的侵袭也有直接作用（18-20）。

MMP-9的循环水平在许多炎症失调疾病中有所升高，包括管腔内血栓的形成（32）、动脉粥样硬化（33）、克罗恩氏病（34）、丙型肝炎病毒感染（35）、结肠直肠癌（36）以及杜氏肌营养不良（37）。MMP-9与TIMP-1的比例在多发性硬化症的血清（38）和囊性纤维化患者的痰（39）中升高，但在巨细胞病毒感染的血清中降低（40）。游离MMP-9的水平、MMP-9与脂质运载蛋白-2/NGAL复合物的水平分别在卵巢癌患者、子宫尿道感染患者的尿液中升高（41, 42）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人MMP-9抗体包被于微孔板上，样品和标准品中的人MMP-9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MMP-9抗体，未结合的抗体被洗去；加入TMB底物溶液，溶液颜色与结合的目标蛋白成正比；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样本，人血清样本，人去血小板血浆，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RD5-10稀释后重新检测；
- 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、试剂盒的储存等。

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。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (ng/mL)	0.833	2.04	11	0.972	2.35	12.2
标准差	0.017	0.039	0.316	0.077	0.184	0.845
CV%	2.0	1.9	2.9	7.9	7.8	6.9

B. 回收率

在样本中掺入检测范围内三个水平的人MMP-9，测定其回收率。

样品类型	平均回收率（%）	范围（%）
细胞培养基*（n=5）	97	85-104
血清*（n=5）	95	91-99
去血小板肝素血浆*（n=5）	96	89-108
唾液*（n=4）	90	74-114
尿液（n=5）	91	82-99

*按照“样品制备”部分的说明，在检测前对样品进行了稀释。

C. 灵敏度

人MMP-9的最低可测剂量（MDD）一般小于0.156 ng/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

此ELISA试剂盒经由R&D Systems®生产的CHO细胞表达的高纯度重组人Pro-MMP-9蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人MMP-9，然后用标准品稀释液RD5-10将样本稀释到检测范围内，测定其线性。

		细胞培养基* (n=5)	血清* (n=5)	去血小板肝素血浆* (n=5)	唾液* (n=4)	尿液 (n=4)
1:2	平均值/期待值（%）	104	103	102	105	101
	范围（%）	101-107	99-107	98-107	103-106	96-109
1:4	平均值/期待值（%）	104	103	102	108	103
	范围（%）	101-106	99-108	99-106	103-109	95-110
1:8	平均值/期待值（%）	102	102	104	111	104
	范围（%）	97-110	96-108	101-110	108-114	94-112
1:16	平均值/期待值（%）	102	102	103	110	97
	范围（%）	96-110	95-108	95-110	109-112	90-109

*在检测前对样品进行了稀释。

F. 样本预值

血清、去血小板血浆、唾液及尿液样本 - 本研究采用表观健康志愿者的上述样本，评估其中人MMP-9的表达/检出情况。所有样本提供者的既往病史信息均不详。

样本类型	可检测均值（ng/mL）	可检测（%）	范围（ng/mL）
血清（n=37）	436	100	169-705
去血小板肝素血浆（n=37）	32	100	13.2-105
唾液（n=4）	201	100	102-543
尿液（n=53）	3.65	45	ND-33.6

ND=未检测到

细胞上清样本-人的外周血单核细胞（5×10^6细胞/mL）培养于含有5%胎牛血清的RPMI1640培养基中，细胞培养基还含有2 mM L-谷氨酰胺、50 μM β-巯基乙醇、100 U/mL青霉素, 100 μg/mL链霉素，部分另加10 μg/mL PHA刺激细胞，在1和5天，取少量未经刺激和刺激后的细胞上清液测定MMP-9含量，结果见下表。

条件	第 1 天 (ng/mL)	第 5 天 (ng/mL)
未刺激	132	33.8
刺激	522	210

G. 特异性

此ELISA法可检测天然及重组人分子量为92 kDa MMP-9前体及82 kDa活性形式MMP-9蛋白，不能测定分子量为65 kDa的MMP-9蛋白。

将以下因子用标准品稀释液RD5-10配置成100 ng/mL或200 ng/mL的浓度来检测与人MMP-9的交叉反应。将200 ng/mL的干扰因子掺入中间范围的重组人MMP-9对照品中，来检测对人MMP-9的干扰。没有观察到明显的交叉反应或干扰。

Recombinant Human		Recombinant mouse
ADAM8	MMP-8	ADAM9
ADAM10	MMP-10	ADAM10
ADAM15	MMP-12	ADAM15
Lipocalin-2/NGAL	MMP-13	Lipocalin-2/NGAL
MMP-1	TACE (ADAM17)	MMP-2
MMP-2	TIMP-2	MMP-3
MMP-3	TIMP-3	MMP-9
MMP-7	TIMP-4	TIMP-1

使用重组人TIMP蛋白干扰检测，浓度≥ 6.25 ng/mL时，观察到有交叉反应。

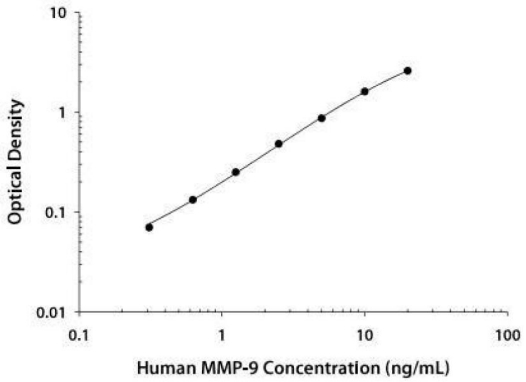
rhTIMP-1 Concentration (ng/mL)	Observed Value (ng/mL)
200	1.241
100	2.026
50	2.920
25	3.651
12.5	4.655
6.25	5.219
0	5.633

使用重组大鼠TIMP-1蛋白干扰检测，当浓度≥ 200 ng/mL时，也观察到有交叉反应。

IV. 实验标准

标准曲线实例

该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。



(ng/mL)	O.D.	Average	Corrected
0	0.040 0.041	0.040	—
0.313	0.111 0.110	0.110	0.070
0.625	0.172 0.170	0.172	0.132
1.25	0.292 0.288	0.290	0.250
2.5	0.532 0.508	0.520	0.480
5	0.910 0.899	0.904	0.864
10	1.612 1.668	1.640	1.600
20	2.607 2.638	2.622	2.582

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
MMP-9 Microplate	包被抗体的96孔聚苯乙烯板，8孔×12条	1块板
MMP-9 Conjugate	酶标记的检测MMP-9抗体	1瓶
MMP-9 Standard	标准品（冻干粉）	2瓶
Assay Diluent RD1-34	检测液RD1-34	1瓶
Calibrator Diluent RD5-10	标准品稀释液RD5-10	3瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）。*可能会随时间变黄。*	1瓶
TMB Substrate	TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Covers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8℃储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>检测液RD1-34</td></tr>
<tr><td>准品稀释液RD5-10</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>现用现配；用后即弃。</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，最多30天*</td></tr>
</table>

*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
- 使用聚丙烯试管稀释标准品和样品
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 水平轨道微孔板振荡仪（0.12英寸轨道直径，500±50 rpm转速）
- 500 mL量筒

D. 注意事项

- 唾液中可检测到MMP-9。采取使用口罩+手套，防止使用试剂盒的过程中有污染。
- 试剂盒中的一些成分含有防腐剂，可能引起皮肤过敏反应。避免吸入。
- 穿戴防护手套、衣服、防护眼镜和脸部保护。操作后彻底洗手。

VI. 实验前准备

A. 样品收集及储存

下面列出的样本收集与储存条件仅为一般性指南。样本稳定性尚未经过评估。

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于≤-20℃冰箱内，避免反复冻融。样本可能需要用标准品稀释液RD5-10稀释。

血清样本：用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 × g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，≤-20℃贮存备用。避免反复冻融。样本可能需要用标准品稀释液RD5-10稀释。

去血小板血浆：以肝素作为抗凝剂，在冰上采集血浆。采集后30分钟内，于1000 × g离心15分钟。建议将血浆在2-8°C下以10,000 × g再次离心10分钟，以完全去除血小板。立即测定，或分装后于≤ -20° C保存。避免反复冻融。样本可能需要用标准品稀释液RD5-10稀释。

注：*由于EDTA和柠檬酸盐具有螯合特性，不建议将其用作本检测中的抗凝剂。*

MMP-9（基质金属蛋白酶-9）在血小板活化时释放。为检测循环血液中的MMP-9水平，应使用去血小板血浆进行测定。需注意，许多血浆制备方案（包括美国临床和实验室标准协会（CLSI）推荐的程序）均存在血小板去除不彻底或导致血小板活化的问题。这可能会使针对血小板所含及活化后释放因子的检测结果出现变异，且缺乏可重复性。

唾液：用Salivette™等收集设备收集唾液，立即检测，或者分装，≤-20℃贮存备用。避免反复冻融。样本可能需要用校准品稀释液RD5-10进行稀释。

注： *唾液采集器不得具有任何酶结合或过滤功能。*

尿液：无菌采集当日第一次晨尿（中段尿），直接排入无菌容器中。离心去除颗粒物。立即检测，或者分装，≤-20℃贮存备用。避免反复冻融。样本可能需要用校准品稀释液RD5-10进行稀释。

B. 样本准备工作

使用聚丙烯试管。

血清、细胞培养上清液及唾液样本在检测前需至少进行100倍稀释。建议的100倍稀释方法为：10 µL样本 + 990 µL校准品稀释液RD5-10。最适稀释倍数应由最终用户自行确定。

去血小板血浆样本需用校准品稀释液RD5-10进行至少40倍稀释。最适稀释倍数应由最终用户自行确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温

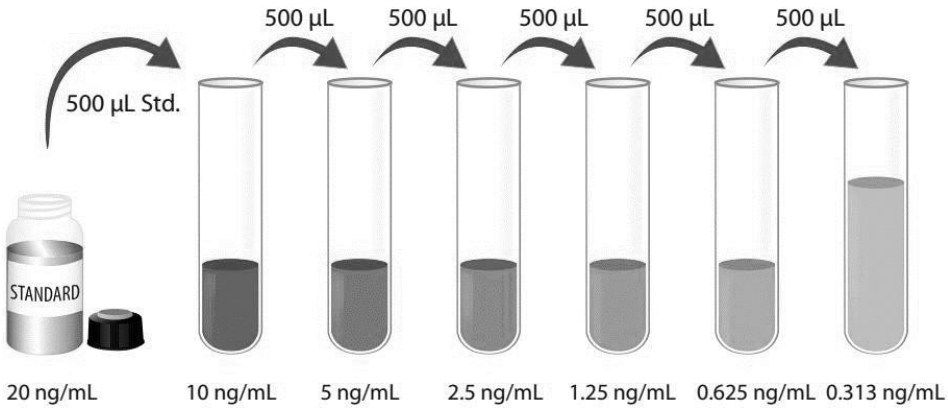
注：*唾液中含高浓度MMP-9。操作时应佩戴口罩和手套，以防试剂盒试剂受到污染。*

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浓缩洗涤液用蒸馏水或去离子水稀释配置成500 mL工作浓度的洗涤液 (1×)。

人MMP-9标准品：复溶体积请参见瓶标签*。使用去离子水或蒸馏水复溶人MMP-9标准品。复溶后得到浓度为20 ng/mL的储备液。在配制稀释液前，需将标准品静置至少15分钟，并伴随温和振荡。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯试管作为标准品稀释管。每个稀释管中加入500µL稀释剂。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。没有稀释的标准品母液可用作标准曲线最高点（20 ng/mL），稀释剂可用作标准曲线零点（0 ng/mL）。



D. 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，配置不同浓度标准品、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

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

注：*唾液中含有高浓度的MMP-9，为避免污染，实验时请带口罩、手套。*

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. 每孔加入100 µL检测液RD1-34；
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，放置震荡仪（0.12英寸）上，500±50转/分钟，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400µL，然后将板内洗涤液吸去。重复操作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酶标检测抗体。用封板膜封住反应孔，**放置震荡仪（0.12英寸）上，500±50转/分钟，室温孵育1小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200µL 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，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，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。如果没有使用双波长校正，结果准确度可能会受影响；
11. **计算结果：**将每个标准品和样品的校正吸光度值 (OD450-OD540/OD570)、复孔读数取平均值，然后减去平均零标准品OD值。使用计算机软件作四参数逻辑(4-PL)曲线拟合创建标准曲线。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。这个过程可生成一个足够使用但不太精确的数据拟合。若样本经过稀释，计算浓度时应乘以稀释倍数。

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

