# PRODUCT INFORMATION & MANUAL

## Human TIMP-1 Valukine™ ELISA

## Catalog Number: VAL213

For the quantitative determination of natural and recombinant human Tissue Inhibitor of Metalloproteinases 1 (TIMP-1) 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 202410.1

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# I. BACKGROUND

Matrix Metalloproteinases (MMPs) are zinc-dependent endopeptidases that catalyze degradation of extracellular matrix proteins, thereby controlling such processes as development, tissue remodeling, wound healing and tumor metastasis (1-3). The activity of MMPs is controlled by regulation of expression and secretion, by proteolytic activation of pro-enzymes and by the Tissue Inhibitors of Metalloproteinases (TIMPs) (4, 5). TIMPs form 1:1, non-covalent complexes with MMPs, blocking access of substrates to the MMP catalytic site. TIMPs are highly specific for MMPs in general but not for any particular MMP. Functional specificity is conferred by other characteristics. TIMP-1 is an inducible protein and TIMP-2 is a constitutive protein and both are soluble and widely distributed. TIMP-3 is restricted to the extracellular matrix and TIMP-4 is largely restricted to cardiac tissue. For reviews on MMPs and TIMPs, see references 1-5.

TIMP-1 is a 184 amino acid residue glycosylated protein, though glycosylation is not necessary for activity (6). It has 12 cysteines (conserved among all TIMPs) that form disulfide bonds in a pattern that gives distinct N- and C-terminal domains (7). The N-terminal domain contains sites that bind to the MMP substrate-binding site (8). Binding of TIMP-1 does not leave a peptide bond in position for proteolysis and is not cleaved (5). The TIMP/MMP complex can dissociate to yield enzyme and active TIMP-1 (9). The C-terminal domain binds to an external site on MMPs, increasing overall affinity (5). TIMP-1 binds with high affinity to the inactive pro-MMP-9, forming a complex in which TIMP-1 retains its ability to inhibit the activity of another active MMP via its N-terminal domain (10).

TIMP-1 is widely synthesized by many cells and tissues (4). Transcription of the TIMP-1 gene is induced by pro-inflammatory cytokines (IL-1, IL-6, OSM, LIF and TNF-α), TGF-β1 and phorbolesters (4, 11). Many physiological functions of TIMP-1 are closely tied to the functions of MMPs, and an improper balance of MMP and TIMP production correlates with pathological conditions such as arthritis, tumor growth and metastasis (4). On the other hand, TIMP-1 was independently discovered as an erythroid potentiating activity (12, 13), an activity that appears to be functionally distinct from MMP inhibitory activity (14). TIMP-1 binds to certain cell lines and is translocated to the nucleus (15). It inhibits apoptosis in B cells (16), further suggesting that it independently functions in multiple ways to support survival and growth of cells in contrast to its function of inhibition of MMPs.

# II. OVERVIEW

## A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human TIMP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human TIMP-1 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 TIMP-1 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 and human saliva.
- 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 (1×) 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) | 0.48 | 1.27 | 6.95 | 0.51 | 1.28 | 6.90 |
| Standard Deviation | 0.02 | 0.05 | 0.35 | 0.02 | 0.05 | 0.34 |
| CV% | 4.2 | 3.9 | 5.0 | 3.9 | 3.9 | 4.9 |

## B. RECOVERY

The recovery of human TIMP-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

| **Sample Type** | **Average % Recovery** | **Range (%)** |
|---|---|---|
| Cell culture media (n=5) | 102 | 94-113 |
| Human Serum* (n=5) | 98 | 87-102 |
| Human Heparin Plasma* (n=5) | 99 | 89-108 |
| Human EDTA Plasma* (n=5) | 99 | 93-108 |
| Human Saliva* (n = 4) | 105 | 89-121 |

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

## C. SENSITIVITY

The minimum detectable dose (MDD) of human TIMP-1 is typically less than 0.08 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 a highly purified NS0-expressed recombinant human TIMP-1 produced at R&D Systems.

## E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human TIMP-1 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

| Dilution | | Cell culture media (n=10) | Human Serum* (n=9) | Human EDTA plasma* (n=9) | Human Heparin plasma* (n=9) | Human Saliva* (n=8) |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 100 | 103 | 102 | 104 | 101 |
| | Range (%) | 97-105 | 100-110 | 98-108 | 100-108 | 94-105 |
| 1:4 | Average % of Expected | 100 | 103 | 103 | 103 | 100 |
| | Range (%) | 96-106 | 98-111 | 96-110 | 95-110 | 96-109 |
| 1:8 | Average % of Expected | 103 | 101 | 100 | 103 | 100 |
| | Range (%) | 89-110 | 93-110 | 92-110 | 94-111 | 95-113 |
| 1:16 | Average % of Expected | 104 | 100 | 100 | 100 | 101 |
| | Range (%) | 91-112 | 93-108 | 93-108 | 85-111 | 87-115 |

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

## F. SAMPLE VALUES

**Human Serum/Plasma/Saliva** - Samples from apparently healthy volunteers were evaluated for the presence of human TIMP-1 in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean (ng/mL) | Range (ng/mL) | Standard Deviation (ng/mL) |
|---|---|---|---|
| Human Serum (n=60) | 190 | 87-524 | 72.1 |
| Human Heparin plasma (n=60) | 84 | 39-279 | 33.1 |
| Human EDTA plasma (n=60) | 98 | 44-304 | 35.4 |
| Human Saliva (n=4) | 121 | 46-208 | 82.3 |

**Cell Culture Supernates** - Human peripheral blood mononuclear cells (5 x $10^6$ cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernate were removed on days 1 and 5 and assayed for levels of natural human TIMP-1.

| Condition | Day 1 (ng/mL) | Day 5 (ng/mL) |
|---|---|---|
| Unstimulated | 76 | 163 |
| Stimulated | 201 | 274 |

## G. SPECIFICITY

This assay recognizes natural and recombinant human (rh) TIMP-1.

The factors listed below were prepared at 200 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human TIMP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

| **Recombinant human：** |
|---|
| MMP-1 |
| MMP-2 |
| MMP-3 |
| TIMP-2 |
| TIMP-3 |
| TIMP-4 |

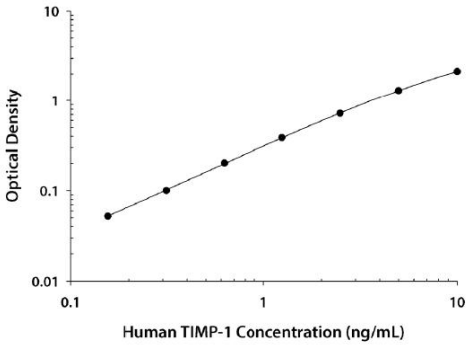
No cross-reactivity was observed with rhMMP-9, but interference was observed at concentrations ≥ 100 ng/mL.

| rhMMP-9 Concentration (ng/mL) | Observed TIMP-1 Value (ng/mL) |
|---|---|
| 200 | 227.6 |
| 100 | 249.8 |
| 50 | 263.4 |
| 25 | 269.8 |
| 12.5 | 287.0 |
| 0 | 276.9 |

# 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 | 0.033<br>0.034 | 0.034 | — |
| 0.156 | 0.086<br>0.087 | 0.086 | 0.052 |
| 0.313 | 0.133<br>0.135 | 0.134 | 0.100 |
| 0.625 | 0.235<br>0.237 | 0.236 | 0.202 |
| 1.25 | 0.419<br>0.423 | 0.421 | 0.387 |
| 2.5 | 0.740<br>0.774 | 0.757 | 0.723 |
| 5 | 1.324<br>1.347 | 1.336 | 1.302 |
| 10 | 2.142<br>2.190 | 2.166 | 2.132 |

# V. KIT COMPONENTS AND STORAGE

## A. MATERIALS PROVIDED

| Parts | Description | Size |
|---|---|---|
| Human TIMP-1 Microplate | 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human TIMP-1 | 1 plate |
| Human TIMP-1 Conjugate | Solution of antibody against human TIMP-1 conjugated to horseradish peroxidase | 1 vial |
| Human TIMP-1 Standard | Recombinant human TIMP-1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume | 1 vial |
| Assay Diluent RD1X | A buffered protein base | 1 vial |
| Calibrator Diluent Concentrate (5×)/ RD5P | A 5× concentrated buffered protein base used to dilute standard and 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="7">Opened/<br>Reconstituted<br>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<br>RD1X</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent<br>Concentrate (5×)/<br>RD5P</td><td>May be stored for up to 1 month at 2-8 °C.*<br>Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</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.
- 100 mL and 500 mL graduated cylinder.
- Horizontal orbital shaker (0.12” orbit) capable of maintaining a speed of 500±50 rpm

## 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.

# 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 (1×).

**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 (1×).

**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 (1×).

**Note:** *Citrate plasma has not been validated for use in this assay.*

*Grossly lipemic samples should not be used in this assay.*

**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 (1×).

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

## B. SAMPLE PREPARATION

All samples recommend a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

## C. REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Note:** *High levels of TIMP-1 are found in saliva. Take necessary precautions (e.g. mask and gloves) to protect kit reagents.*

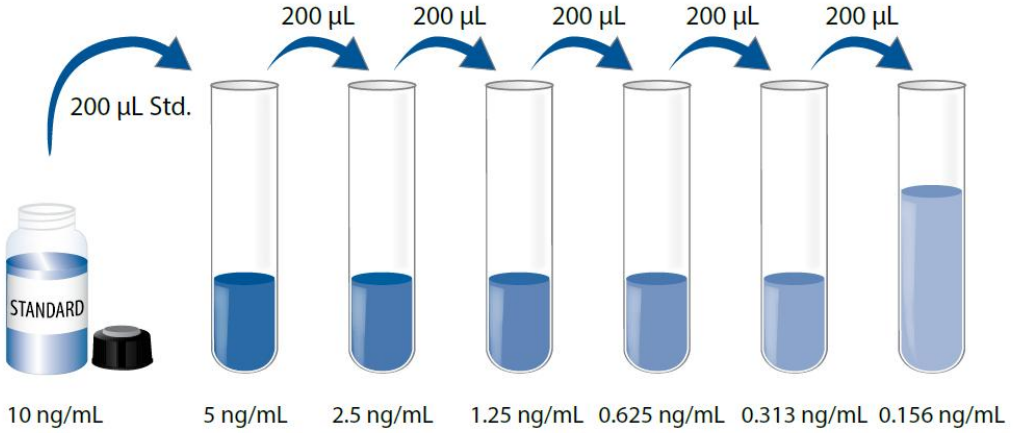
**Wash Buffer (1×)** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

**Calibrator Diluent (1×)** - Use deionized or distilled water to prepare Calibrator Diluent (1×).

**Human TIMP-1 Standard - Refer to the vial label for the reconstitution volume***. Reconstitute the Human TIMP-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10 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.

Pipette 200 µL of Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard stock serves as the high standard (10 ng/mL). Calibrator Diluent (1×) 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 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 levels of TIMP-1 are found in saliva. Take necessary precautions (e.g. mask and gloves) to protect kit reagents.*

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 RD1X to each well. *Assay Diluent RD1X may contain a crystalline precipitate. Mix well before and during use.*
4. Add 50 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital 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 two times for a total of three 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 TIMP-1 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500±50 rpm.**
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. 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 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 human TIMP-1 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.

## VIII. REFERENCES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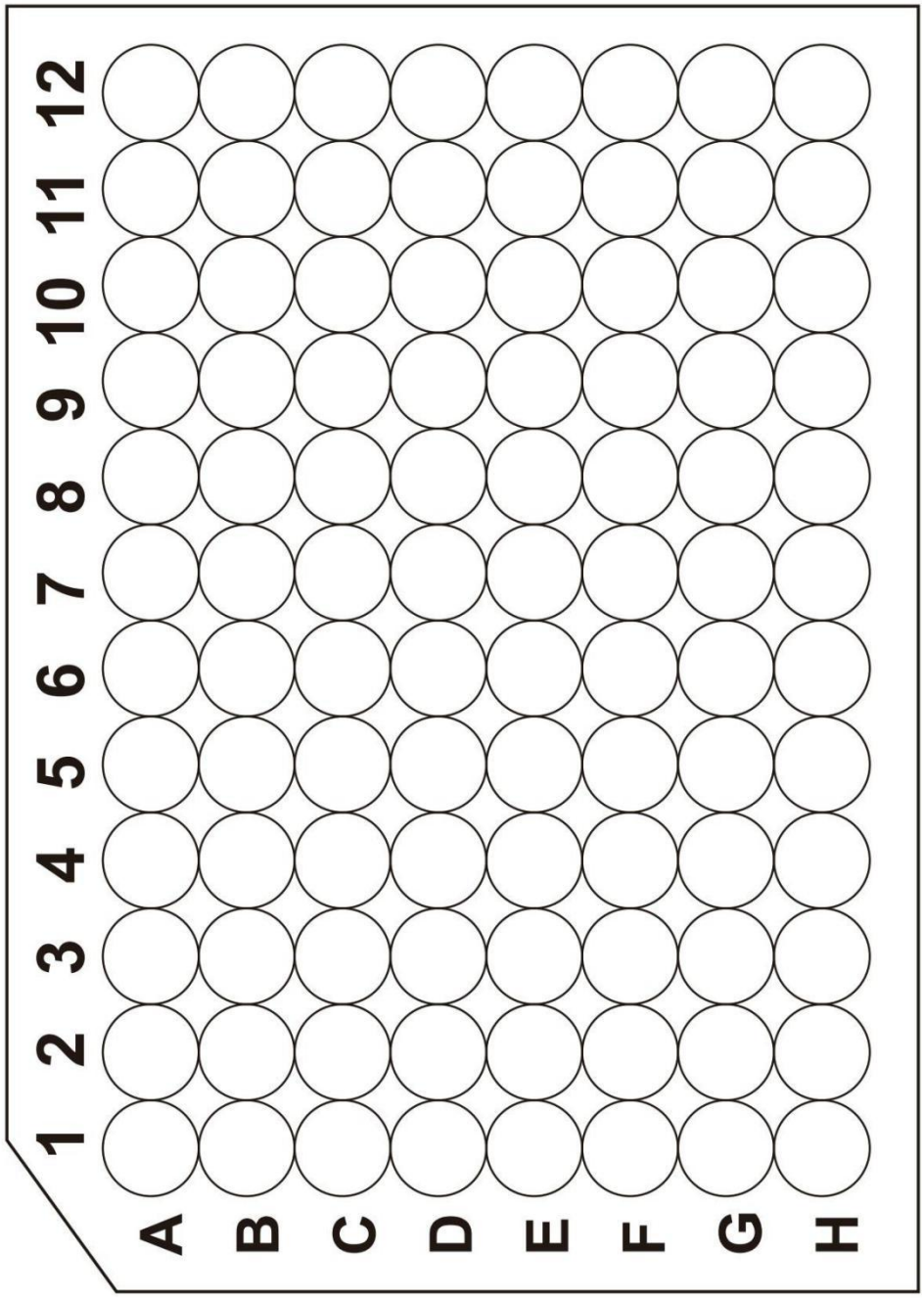
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



# 产品信息及操作手册

## 人 TIMP-1 Valukine™ ELISA 试剂盒

**目录号：VAL213**

适用于定量检测天然和重组人金属蛋白酶组织抑制因子 1 (TIMP-1)的浓度

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# 目录

# I. 背景

基质金属蛋白酶（Matrix Metalloproteinases，MMPs）是一种锌依赖性内肽酶，可催化细胞外基质蛋白的降解，从而控制发育、组织重塑、伤口愈合和肿瘤转移等过程 (1-3)。MMPs 的活性受表达和分泌调节、原酶的蛋白水解活化以及金属蛋白酶组织抑制因子（TIMPs）的控制（4，5）。TIMPs 与 MMPs 形成 1:1 的非共价复合物，阻止底物进入 MMP 催化位点。TIMPs 对大多数的 MMP 具有高度特异性，该高度特异性并不针对特定的 MMP。功能特异性由其他特征决定。TIMP-1 是一种诱导型蛋白质，TIMP-2 是一种组成型蛋白质，两者都是可溶性的，分布广泛。TIMP-3 局限于细胞外基质，而 TIMP-4 则主要局限于心脏组织。有关 MMPs 和 TIMPs 的综述，请参阅参考文献 1-5。

TIMP-1 是一种具有 184 个氨基酸残基的糖基化蛋白质，但糖基化并不是其活性的必要条件（6）。它有 12 个半胱氨酸（所有 TIMPs），这些半胱氨酸形成二硫键，从而形成不同的 N 端和 C 端结构域（7）。N 端结构域包含与 MMP 底物结合位点与 MMP 底物结合位点结合（8）。与 TIMP-1 结合后，肽键不会被蛋白水解，也不会被裂解（5）。TIMP/MMP 复合物可以解离，生成酶和激活 TIMP-1（9）。C 端结构域与 MMP 的外部位点结合，增加了整体亲和力（5）。TIMP-1 与无活性 Pro-MMP-9 具有高亲和力结合，形成一个复合物，其中 TIMP-1 在其 N 末端结构域通过抑制有活性的其他 MMP 的活性来保留自己的活性（10）。

许多细胞和组织都能广泛合成 TIMP-1（4）。促炎细胞因子（IL-1，IL-6，OSM，LIF 和 TNF-α），TGF-β1 和佛波酯会诱导 TIMP-1 基因的转录（4，11）。TIMP-1 的许多生理功能与 MMP 的功能密切相关，而 MMP 和 TIMP 生成的不平衡与关节炎、肿瘤生长和转移等病理情况相关（4）。另一方面，TIMP-1 被发现具有红细胞增效活性（12，13），这种活性在功能上与 MMP 抑制活性不同（14）。TIMP-1 与某些细胞系结合，并转运至细胞核（15）。它能抑制 B 细胞的凋亡（16），这进一步表明，与抑制 MMPs 的功能相比，它能以多种方式独立地支持细胞的存活和生长。

# II. 概述

## A. 检测原理

本实验采用双抗体夹心ELISA法。抗人TIMP-1抗体包被于微孔板上，样品和标准品中的人TIMP-1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TIMP-1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样本，人血清、人血浆和人唾液；
- 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（1×）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。

| | 板内精确度 | | | 板间精确度 | | |
|---|---|---|---|---|---|---|
| 样本 | 1 | 2 | 3 | 1 | 2 | 3 |
| 平均值 (ng/mL) | 0.48 | 1.27 | 6.95 | 0.51 | 1.28 | 6.90 |
| 标准差 | 0.02 | 0.05 | 0.35 | 0.02 | 0.05 | 0.34 |
| CV% | 4.2 | 3.9 | 5.0 | 3.9 | 3.9 | 4.9 |

### B. 回收率

不同类型样本中掺入检测范围内不同水平的人TIMP-1，测定其回收率。

| 样本类型 | 平均回收率 (%) | 范围 (%) |
|---|---|---|
| 细胞培养基 (n=5) | 102 | 94-113 |
| 人血清* (n=5) | 98 | 87-102 |
| 人肝素血浆* (n=5) | 99 | 89-108 |
| 人 EDTA 血浆* (n=5) | 99 | 93-108 |
| 人唾液* (n=4) | 105 | 89-121 |

*样品在分析前按照样品制备部分的指示进行稀释。

### C. 灵敏度

人 TIMP-1 的最小可检测剂量（MDD）通常小于 0.08 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

该免疫测定法以R&D Systems生产的高纯度的NS0表达的重组人TIMP-1校正。

## E. 线性

不同的样本中含有或掺入高浓度的人TIMP-1，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

| 稀释倍数 | | 细胞培养基 (n=10) | 人血清* (n=9) | 人EDTA血浆* (n=9) | 人肝素血浆* (n=9) | 人唾液* (n=8) |
|---|---|---|---|---|---|---|
| 1:2 | 平均值/期待值（%） | 100 | 103 | 102 | 104 | 101 |
| | 范围 (%) | 97-105 | 100-110 | 98-108 | 100-108 | 94-105 |
| 1:4 | 平均值/期待值（%） | 100 | 103 | 103 | 103 | 100 |
| | 范围 (%) | 96-106 | 98-111 | 96-110 | 95-110 | 96-109 |
| 1:8 | 平均值/期待值（%） | 103 | 101 | 100 | 103 | 100 |
| | 范围 (%) | 89-110 | 93-110 | 92-110 | 94-111 | 95-113 |
| 1:16 | 平均值/期待值（%） | 104 | 100 | 100 | 100 | 101 |
| | 范围 (%) | 91-112 | 93-108 | 93-108 | 85-111 | 87-115 |

*样品在分析前按照样品制备部分的指示进行稀释。

## F. 样本预值

**人血清/血浆/唾液**–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TIMP-1。本研究中使用的供体没有病史。

| 样本类型 | 平均值 (ng/mL) | 范围 (ng/mL) | 标准偏差 (ng/mL) |
|---|---|---|---|
| 人血清 (n=60) | 190 | 87-524 | 72.1 |
| 人肝素血浆 (n=60) | 84 | 39-279 | 33.1 |
| 人EDTA血浆 (n=60) | 98 | 44-304 | 35.4 |
| 人唾液 (n=4) | 121 | 46-208 | 82.3 |

**细胞培养上清**-人外周血单核细胞（5×10⁶个细胞/mL）在RPMI培养基中培养，该培养基补充了5%胎牛血清、50 μMβ-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。细胞在不刺激或用10 μg/mL PHA刺激的情况下培养。在第1天和第5天取培养上清液，测定天然人TIMP-1水平。

| 条件 | 1 天 (ng/mL) | 5 天 (ng/mL) |
|---|---|---|
| 未刺激 | 76 | 163 |
| 刺激 | 201 | 274 |

## G. 特异性

检测方法识别天然和重组人TIMP-1。

以下列出的因子在标准品稀释液（1×）中以 200 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TIMP-1对照品中以200 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：** |
|---|
| MMP-1 |
| MMP-2 |
| MMP-3 |
| TIMP-2 |
| TIMP-3 |
| TIMP-4 |

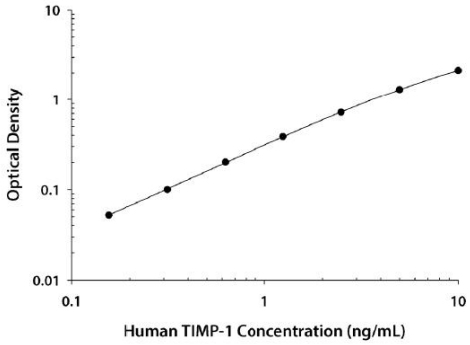
未观察到与rhMMP-9的交叉反应，但在浓度≥100 ng/mL时观察到干扰。

| rhMMP-9浓度（ng/mL） | TIMP-1检测值（ng/mL） |
|---|---|
| 200 | 227.6 |
| 100 | 249.8 |
| 50 | 263.4 |
| 25 | 269.8 |
| 12.5 | 287.0 |
| 0 | 276.9 |

# IV. 实验

## 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 | 0.033<br>0.034 | 0.034 | — |
| 0.156 | 0.086<br>0.087 | 0.086 | 0.052 |
| 0.313 | 0.133<br>0.135 | 0.134 | 0.100 |
| 0.625 | 0.235<br>0.237 | 0.236 | 0.202 |
| 1.25 | 0.419<br>0.423 | 0.421 | 0.387 |
| 2.5 | 0.740<br>0.774 | 0.757 | 0.723 |
| 5 | 1.324<br>1.347 | 1.336 | 1.302 |
| 10 | 2.142<br>2.190 | 2.166 | 2.132 |

## V. 试剂盒组成及储存

### A. 试剂盒组成

| 组成 | 描述 | 规格 |
| --- | --- | --- |
| Human TIMP-1 Microplate | 包被抗人TIMP-1抗体的96孔聚苯乙烯板，8孔× 12条 | 1块板 |
| Human TIMP-1 Conjugate | 酶标检测抗人TIMP-1抗体 | 1瓶 |
| Human TIMP-1 Standard | 重组人TIMP-1标准品（冻干），参考瓶身标签进行重溶 | 1瓶 |
| Assay Diluent RD1X | 检测液 | 1瓶 |
| Calibrator Diluent Concentrate (5×)/ RD5P | 浓缩的标准品稀释液（5×），用于稀释标准品和样品 | 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

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</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>2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1X</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（5×）/ RD5P</td><td>2-8℃储存，最多 30 天*<br>请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</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 °C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
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 100 mL和500 mL量筒
- 水平振荡器（0.12”轨道），转速：500±50 rpm

## 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。

# 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（1×）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。避免反复冻融循环。样本可能需要用标准品稀释液（1×）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（1×）稀释。

***注：****柠檬酸盐血浆在本检测中未经验证。*

*严重脂血样本不得用于本检测。*

**唾液** - 使用唾液采集器，如Salivette或等效设备采集唾液。立即检测或分装后于≤-20 °C储存。避免反复冻融循环。样品可能需要用标准品稀释液（1×）进行稀释。

***注：****唾液收集器不得具有任何酶结合或过滤功能。*

## B. 样品准备

所有样本建议进行 100 倍稀释。建议的 100 倍稀释量为：10 µL 的样本+990 µL 的标准品稀释液（1×）。最佳稀释倍数应由用户自行确定。

## 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TIMP-1含量较高。请采取必要预防措施（例如戴口罩和手套）以保护试剂盒试剂。*

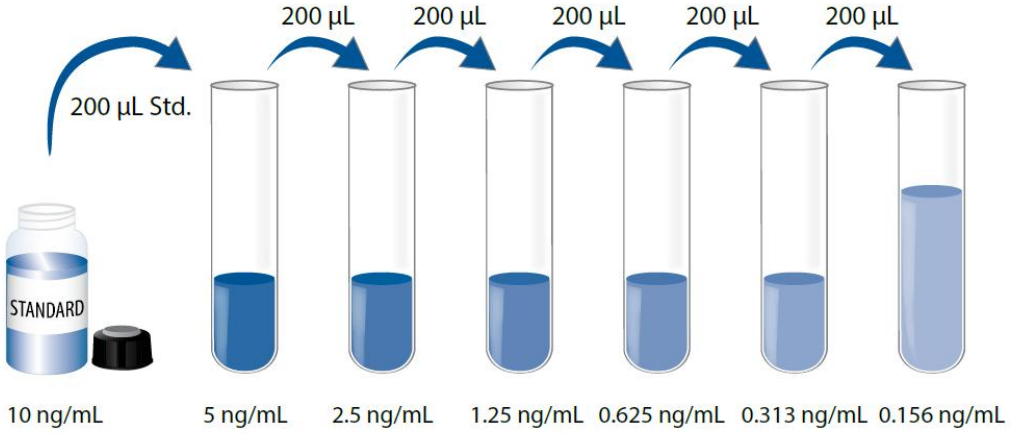
**洗涤液（1×）：**从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

**标准品稀释液（1×）：**使用去离子水或蒸馏水制备标准品稀释液（1×）。

**人TIMP-1标准品：**重溶体积请参考瓶身标签*，用去离子水或蒸馏水重构人TIMP-1标准品，得到浓度为10 ng/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

用移液管将200 µL标准品稀释液（1×）移入管中。使用储备母液溶液稀释（如下）。在下次转移之前，将每个管彻底混合。未稀释的标准品作为最高标准品（10 ng/mL）。标准品稀释液（1×）作为标准品零点（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带至室温。建议对所有标准品和样品进行复孔检测。**

***注：****唾液中TIMP-1含量较高。请采取必要预防措施（例如戴口罩和手套）以保护试剂盒试剂。*

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RD1X。*检测液RD1X中可能含有结晶沉淀。使用前和使用过程中请充分混合。*
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，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2次，共洗3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TIMP-1酶标检测抗体。用封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500±50 rpm，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.），使用计算机软件作四参数逻辑（4-PL）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轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人TIMP-1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。该程序将产生足够但不太精确的数据拟合。

    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