



PRODUCT INFORMATION & MANUAL

Human Osteoprotegerin/TNFRSF11B Valukine™ ELISA

VAL174

For the quantitative determination of natural and recombinant
human Osteoprotegerin 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

Version202302.1

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I. BACKGROUND

Human Osteoprotegerin (OPG), also known as Tumor necrosis factor receptor superfamily member 11B (TNFRSF11B), is a 60kDa glycoprotein member of the tumor necrosis factor receptor superfamily (1-4). This cytokine is a homodimer expressed and secreted by multiple adult tissues such as the lung, liver (5, 6), spleen (7), prostate (8), ovary (9) and bone marrow (10). It plays a critical function in bone homeostasis by acting as a decoy receptor for TNFSF11/RANKL and for the receptor activator of nuclear factor-kappa-B ligand (11, 12). OPG therefore decreases bone resorption by inhibiting osteoclast differentiation and activation of osteoclast and by stimulating osteoclast apoptosis. OPG deficiency has been found in Juvenile Paget disease, a rare autosomal recessive osteopathy that presents in infancy and early childhood characterized by rapidly remodeling woven bone, osteopenia, debilitating fractures and deformities due to accelerated bone remodeling (13, 14). OPG may also play a role in arterial calcification and high levels of OPG have been found to be an independent risk factor for cardiovascular disease and vascular mortality (15, 16).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A mouse antibody specific for human OPG has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any OPG present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human OPG is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB Substrate solution is added to the wells and color develops in proportion to the amount of OPG 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 and human serum.
- ◆ 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 Reagent Diluent (1x) 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 twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	1063.6	250.2	58.1	1043.3	244.7	59.1
Standard Deviation	45.2	10.8	2.9	56.8	11.8	3.2
CV%	4.3	4.3	5.0	5.4	4.8	5.3

B. RECOVERY

The recovery of human OPG spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 81.7-105.2% with an average of 96.5%.

The recovery of human OPG spiked to different levels throughout the range of the assay in serum was evaluated. The recovery ranged from 88.7-102.6% with an average of 95.4%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human OPG is typically less than 2.05 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 Sf 21-expressed recombinant human OPG/Fc Chimera produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human OPG in various matrices and diluted with Reagent Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	93.4	89.2-97.6
1:4	94.9	89.4-99.4
1:8	98.1	93.9-102.2
1:16	93.7	85.6-103.3

F. SAMPLE VALUES

Cell Culture Supernates - MCF-7 cells (4×10^5 cells/mL) were cultured in DMEM/F12 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured for 48h, the culture supernatant was obtained by centrifugation. Aliquots of the culture supernates were removed. An aliquot of the cell culture supernate was removed, assayed for human OPG, and measured 2362 pg/mL.

Serum - Five human serum samples were evaluated for the presence of human OPG in this assay. All samples measured ranged from 3501 to 4138 pg/mL with an average of 3871 pg/mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

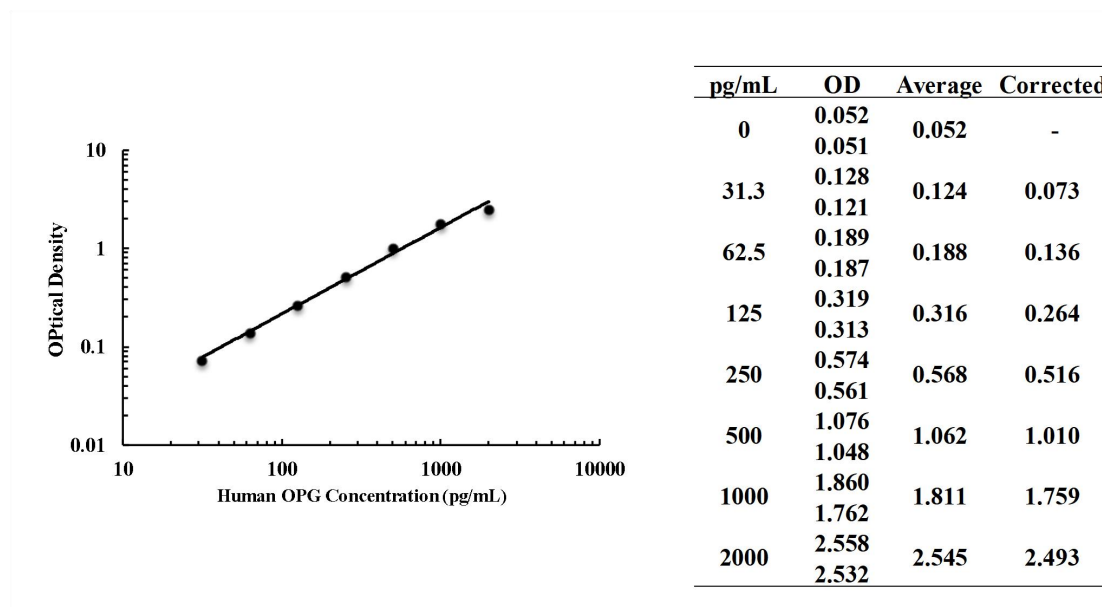
Recombinant human
CD40
TNF RI
TNF RII

Recombinant human TRANCE and recombinant mouse TRANCE do not cross-react in this assay but do interfere at concentrations > 250 pg/mL and 500 pg/mL respectively. A sample containing 6250 pg/mL of recombinant mouse OPG reads as 1062 pg/mL (17% cross-reactivity).

IV. EXPERIMENT

EXAMPLE STANDARD

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



V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human OPG Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human OPG.	1 plate
Human OPG Detection Antibody	Biotinylated human OPG antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Human OPG Standard	Recombinant human OPG in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute standard, samples and HRP.	1 vial
Detection Antibody Diluent Concentrate (5×)	A 5× concentrated buffered protein base used to dilute detection antibody.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution.	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP A	May be stored for up to 1 month at 2-8 °C.*
	Diluted Wash Solution	
	TMB Substrate	
	Stop Solution	
	Standard	Prepare fresh for each assay. Standards may be stored for up 1 month at -20°C.*
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	Reagent Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.
	Detection Antibody Diluent Concentrate (5×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.
	Microplate Wells	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.*

* 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.

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 protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernate - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples require dilution with Reagent Diluent (1 $\times$).

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 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples require dilution with Reagent Diluent (1 $\times$).

B. SAMPLE PREPARATION

Cell Culture Supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 100 μL of sample + 100 μL of Reagent Diluent (1 $\times$).

Serum samples require a 10-fold dilution. A suggested 10-fold dilution is 10 μL of sample + 90 μL of Reagent Diluent (1 $\times$).

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

Wash Buffer (1 $\times$) - 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 $\times$) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 $\times$).

Reagent Diluent (1 $\times$) - Use deionized or distilled water to prepare Reagent Diluent (1 $\times$).

Detection Antibody Diluent (1 $\times$) - Use deionized or distilled water to prepare Detection Antibody Diluent (1 $\times$).

Detection Antibody - **Centrifuge briefly before opening. Reconstitution volume refer to vial label with Reagent Diluent (1 $\times$).** Aliquot and store if needed. Dilute stock solution in Detection Antibody Diluent (1 $\times$) to the working concentration of 200 ng/mL. Prepare at least 15 minutes prior to use.

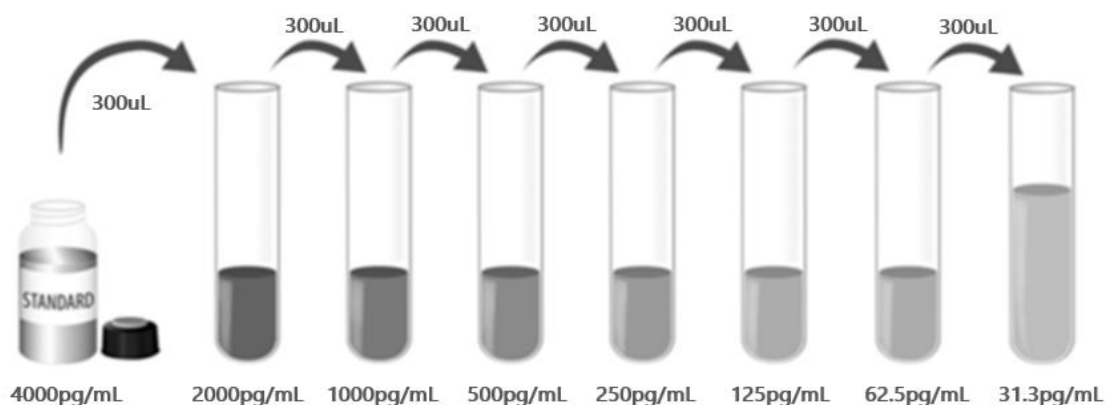
Streptavidin-HRP A (1 $\times$) - **Centrifuge briefly before opening.** Dilute to the working concentration specified on the vial label using Reagent Diluent (1 $\times$).

Human OPG Standard - **Centrifuge briefly before opening. Refer to the vial label**

for reconstitution volume*. This reconstitution produces a stock solution of 4000 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 300 μ L of Reagent Diluent (1 $\times$) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The Reagent Diluent (1 $\times$) serves as the zero standard (0 pg/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

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

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 μ L of standard, or 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. (Samples may require dilution. See Sample Preparation section.)
4. 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 μ L) 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.
5. Add 100 μ L of the Detection Antibody diluted in Detection Antibody Diluent (1x), to each well. Cover with a new adhesive strip and incubate for **2 hours at room temperature**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μ L of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for **20 minutes at room temperature. Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μ L of TMB Substrate to each well. Incubate for **20 minutes at room temperature. Avoid placing the plate in direct light.**
10. Add 50 μ L 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.
11. 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.

12. **CALCULATION OF RESULTS:** Average the duplicate readings for each standard, control, 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 human OPG 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

1. Holen I, Shipman CM. (2006) Clin Sci (Lond). 110:279-291.
2. Cheng K. et al. (2013) J.Genet Syndr Gene Ther. 4:1000153.
3. D.M. Anderson. et al. (1997) Nature. 390:175-179.
4. Wright HL. et al. (2009) Curr Rev Musculoskelet Med. 2:56-64.
5. Adhyatmika A, et al. (2020) Pharmaceutics. 12:471.
6. Adhyatmika, A. et al. (2022) Cell Physiol Biochem. 56:28.
7. Kondo H. et al. (2002) J Bone Miner Res. 17:1667.
8. Chen G. et al. (2006) Cancer. 107:289-98.
9. Zhang Y. et al. (2020) Int J Immunopathol Pharmacol. 34:2058738420941762.
10. Zhang L. et al. (2016) Cell Physiol Biochem. 40:681.
11. Li H. et al. (2018) Autophagy. 14:1726.
12. Kenkre JS, Bassett J. (2018) Ann Clin Biochem. 55:308.
13. Whyte MP. et al. (2014) Bone. 468:153.
14. Gottesman GS. et al. (2016) Am J Med Genet A. 170A:978.
15. Venuraju SM. et al. (2010) J Am Coll Cardiol. 55:2049.
16. Ndip A. et al. (2011) Diabetes. 60:2187.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

1	2	3	4	5	6	7	8	9	10	11	12								
A	B	C	D	E	F	G	H												



产品信息及操作手册

人 Osteoprotegerin/TNFRSF11B Valukine™ ELISA 试剂盒

目录号: **VAL174**

适用于定量检测天然和重组人 Osteoprotegerin 的浓度

科研专用，不可用于临床诊断

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I. 背景

人骨保护素(Osteoprotegerin, OPG), 又称肿瘤坏死因子受体超家族成员 11B(Tumor necrosis factor receptor superfamily member 11B, TNFRSF11B), 是肿瘤坏死因子受体超家族中其中一个 60 kDa 大小的糖蛋白成员 (1-4)。这种细胞因子是由人肺、肝、脾、前列腺、卵巢和骨髓等多种组织表达和分泌的同源二聚体 (5-10)。它作为 TNFSF11/RANKL 的诱导受体和核因子- κ B 受体的活化因子配体在骨稳态中发挥重要作用 (11, 12)。因此, OPG 通过抑制破骨细胞分化和破骨细胞活化以及刺激破骨细胞凋亡减少骨吸收。在青少年 Paget 病中发现了 OPG 缺乏症, 这是一种罕见的常染色体隐性遗传性骨病, 表现在婴儿期和幼儿期, 其特征是快速重塑编织骨, 骨质减少, 由于加速骨重塑而导致的衰弱性骨折和畸形 (13, 14)。OPG 也可能在动脉钙化中发挥作用, 研究发现 OPG 的高表达水平是心血管疾病和血管死亡率的独立危险因素 (15, 16)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。小鼠抗人OPG抗体包被于微孔板上，样品和标准品中的人OPG会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人OPG检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（Streptavidin-HRP）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和人血清样本；
- ◆ 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，应将样本用试剂稀释液（1×）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20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 (pg/mL)	1063.6	250.2	58.1	1043.3	244.7	59.1
标准差	45.2	10.8	2.9	56.8	11.8	3.2
CV%	4.3	4.3	5.0	5.4	4.8	5.3

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人OPG，测定其回收率。回收率范围在81.7-105.2%，平均回收率在96.5%。

在血清样本中掺入检测范围内不同水平的人OPG，测定其回收率。回收率范围在88.7-102.6%，平均回收率在95.4%。

C. 灵敏度

人OPG的最低可测剂量（MDD）一般小于 2.05 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

此ELISA试剂盒经由R&D Systems生产的高纯度Sf 21表达的重组人OPG / Fc嵌合体所校正。

E. 线性

在不同类型样本中掺入高浓度的人OPG，然后用样品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数	平均值/期待值 (%)	范围 (%)
1:2	93.4	89.2-97.6
1:4	94.9	89.4-99.4
1:8	98.1	93.9-102.2
1:16	93.7	85.6-103.3

F. 样本预值

细胞上清样本 - MCF-7细胞 (4×10^5 cells/mL) 用含10 %胎牛血清、100 U/mL青霉素、100 µg/mL硫酸链霉素的DMEM/F12培养基培养, 细胞培养48h, 离心, 得到培养上清。取细胞培养上清液, 测定人细胞培养上清样本中人OPG的水平, 检测值为2362 pg/mL。

血清样本 - 使用本试剂盒检测了5份人血清样本中OPG的水平。5份样本的检测值在3501 - 4138 pg/mL之间, 平均值为3871 pg/mL。

G. 特异性

对50ng/mL浓度的下列因子进行了测定, 未显示交叉反应性或干扰。

Recombinant human
CD40
TNF RI
TNF RII

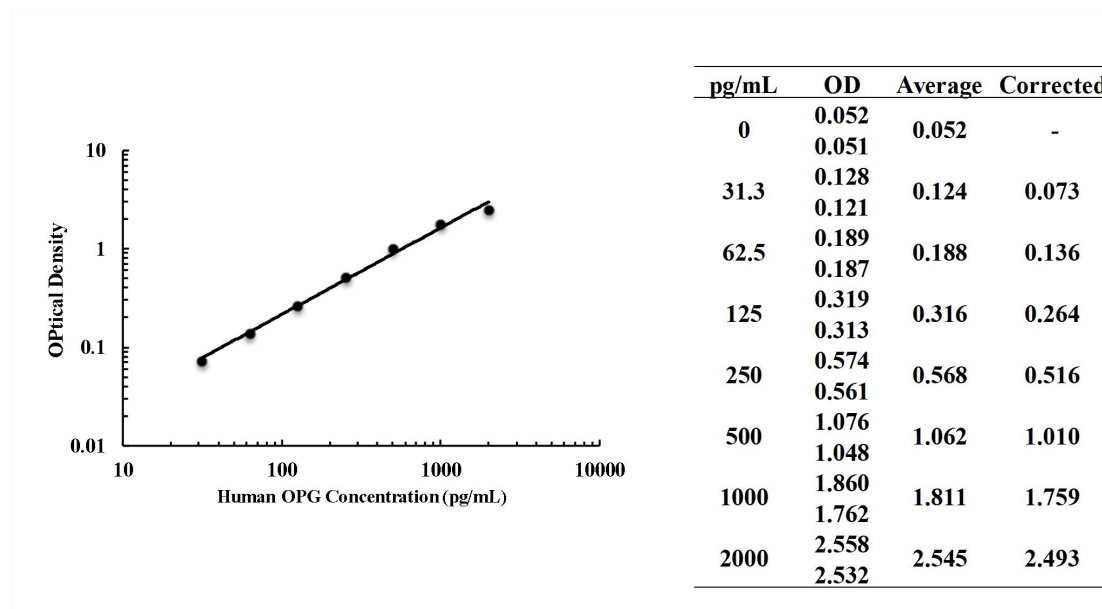
重组人 TRANCE 和重组小鼠 TRANCE 在本试验中不发生交叉反应, 但分别在浓度>250 pg/mL 和 500 pg/mL 时产生干扰。

含有 6250 pg/mL 重组小鼠 OPG 的样品读数为 1062 pg/mL (17%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。



V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human OPG Microplate	包被小鼠抗人 OPG 抗体的 96 孔聚苯乙烯板, 8 孔×12 条	1 块板
Human OPG Detection Antibody	生物素化的人 OPG 检测抗体, 冻干粉, 参考瓶身标签进行重溶	1 瓶
Human OPG Standard	标准品 (冻干粉), 参考瓶身标签进行重溶	2 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液 (10×) 用于稀释标准品, 样本和 HRP	1 瓶
Detection Antibody Diluent Concentrate (5×)	浓缩的检测抗体稀释液 (5×) 用于稀释检测抗体	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP A	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液 (25×)	1 瓶
TMB Substrate	TMB 底物溶液	1 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℃储存；请在试剂盒有效期内使用	
已打开，稀释 或重溶的试剂	链霉亲和素-HRP A	2-8℃储存，最多30天*
	洗涤缓冲液 (1×)	
	TMB底物溶液	
	终止液	
	标准品	使用时新鲜配制* 标准品-20℃储存，最多30天*
	检测抗体	分装，-20℃储存，最多30天*
	试剂稀释液 (10×)	2-8℃储存，最多30天* 请每次使用新鲜配制的1×试剂稀释液
	检测抗体稀释液 (5×)	2-8℃储存，最多30天* 请每次使用新鲜配制的1×检测抗体稀释液
	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℃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

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。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

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。样本需要用试剂稀释液（1×）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 x 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。样本需要用试剂稀释液（1×）稀释。

B. 样本准备工作

细胞培养上清样本需要用试剂稀释液（1×）2倍稀释后进行检测，即100 μL样本+100 μL试剂稀释液（1×）。

血清样本需要用试剂稀释液（1×）10倍稀释后进行检测，即10 μL样本+90 μL试剂稀释液（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温

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制成500 mL工作浓度的洗涤液（1×）。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配制成试剂稀释液（1×）。

检测抗体稀释液（1×）：使用蒸馏水或去离子水稀释配制成检测抗体稀释液（1×）。

检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶。再用检测抗体稀释液（1×）稀释至工作浓度200 ng/mL，至少在使用前15分钟准备。

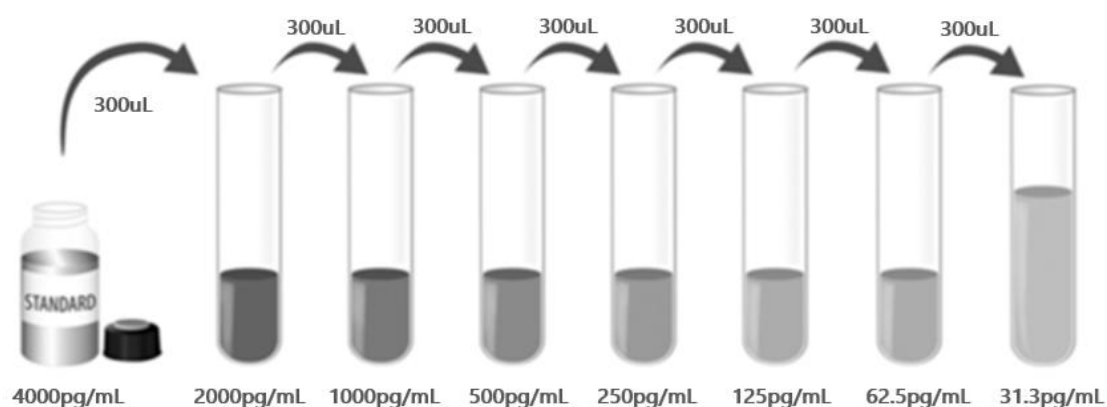
链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

人OPG标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签*，得到浓度为4000 pg/mL标准品母液。轻轻震荡至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300 μL试剂稀释液（1×）。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。2000 pg/mL的标准品可用作标准曲线最高点，试剂稀释液（1×）可用作标准曲线零点（0 pg/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

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. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，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；（样本需要稀释，详情参见样本制备部分。）
4. 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；
5. 在每个微孔内加入100 μL 配制好的检测抗体。用封板膜封住反应孔，**室温孵育2小时**；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 μL 稀释好的链霉亲和素-HRP A 工作液。用封板膜封住反应孔，**室温孵育20分钟，注意避光**；
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 μL TMB底物溶液，**室温孵育20分钟。注意避光**；
10. 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；
11. 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；
12. **计算结果：**将每个标准品和样品的校正吸光度值($\text{OD}_{450}-\text{OD}_{540}/\text{OD}_{570}$)，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。通过样本的OD值，可从标准曲线上得到样本中人OPG的浓度。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

1. Holen I, Shipman CM. (2006) Clin Sci (Lond). 110:279-291.
2. Cheng K. et al. (2013) J. Genet Syndr Gene Ther. 4:1000153.
3. D.M. Anderson. et al. (1997) Nature. 390:175-179.
4. Wright HL. et al. (2009) Curr Rev Musculoskelet Med. 2:56-64.
5. Adhyatmika A, et al. (2020) Pharmaceutics. 12:471.
6. Adhyatmika, A. et al. (2022) Cell Physiol Biochem. 56:28.
7. Kondo H. et al. (2002) J Bone Miner Res. 17:1667.
8. Chen G. et al. (2006) Cancer. 107:289-98.
9. Zhang Y. et al. (2020) Int J Immunopathol Pharmacol. 34:2058738420941762.
10. Zhang L. et al. (2016) Cell Physiol Biochem. 40:681.
11. Li H. et al. (2018) Autophagy. 14:1726.
12. Kenkre JS, Bassett J. (2018) Ann Clin Biochem. 55:308.
13. Whyte MP. et al. (2014) Bone. 468:153.
14. Gottesman GS. et al. (2016) Am J Med Genet A. 170A:978.
15. Venuraju SM. et al. (2010) J Am Coll Cardiol. 55:2049.
16. Ndip A. et al. (2011) Diabetes. 60:2187.

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

