PRODUCT INFORMATION & MANUAL

Human IL-8/CXCL8 Valukine™ ELISA

VAL103

For the quantitative determination of natural and recombinant human IL-8/CXCL8 concentrations

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

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Novus kits are guaranteed for 3 months from date of receipt

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I. BACKGROUND

Interleukin-8 (IL-8; also known as GCP-1, NAP-1, and CXCL8) is a heparin-binding, 8-9 kDa member of the alpha, or CXC family of chemokines. Currently, there are 15 human CXC family members that generally range from 8-12 kDa in size. The majority are found on human chromosome 4, all contain a typical three β-sheet/one α-helix structure, and most show an N-terminal GluLeuArg (ELR) tri-peptide motif (1-3). Human IL-8 is synthesized as a 99 amino acid (aa) precursor that contains a 20 aa signal sequence plus a 79 aa mature region (4-6). It circulates as a monomer, homodimer, and heterodimer with CXCL4/PF4. The monomer is considered the most bioactive, while the heterodimer is proposed to potentiate PF4 activity (7-10). Mature human IL-8 shares 65% and 70% aa sequence identity with porcine and canine IL-8, respectively (11, 12). There is no IL-8 gene counterpart in rodents. Multiple isoforms of IL-8 exist that are generated through both alternative splicing and differential proteolytic processing. Alternative splicing occurs at the C-terminus where there is an

11 aa substitution for amino acids 92-99 (13). Proteolytic processing is likely a cell-specific event and results in IL-8 N-terminal truncation. For example, fibroblasts and endothelial cells cleave amino acids 21 and 22, generating IL-8 (1-7), while monocytes and lymphocytes cleave amino acids 21-25, generating IL-8 (6-7). These short forms generally show increased bioactivity, particularly towards the CXCR1 IL-8 receptor (6, 14). Approximately 15% of IL-8 also undergoes citrullination on Arg27 of the precursor. This creates an IL-8 with an increased half-life and ability to induce leukocytosis (15, 16). A wide variety of cells secrete IL-8, and these include monocytes and neutrophils (17), fibroblasts and keratinocytes (18), mast cells (19), visceral smooth muscle cells (20), dendritic cells (21), type II Greater alveolar cells (22), and endothelial cells (23).

There are two G-protein-coupled receptors for IL-8, termed CXCR1/IL-8RA and CXCR2/IL-8RB, that share 77% aa sequence identity (24). CXCR1 is 45-50 kDa in size and used almost exclusively by IL-8. CXCR2 is 35-40 kDa in size and used by almost all CXC chemokines (25, 26). Both CXCR1 and CXCR2 constitutively homodimerize, and this appears to be their functional configuration. When expressed on the same cell, heterodimerization will also occur, but this configuration undergoes disassembly following IL-8 binding (27). CXCR2 responds to low concentrations of IL-8 and is principally associated with chemotaxis and MMP-9 release. CXCR1, by contrast, responds to high concentrations of IL-8 and is associated with respiratory burst and phospholipase D2 activation (26). Thus, and in the case of neutrophils, CXCR2 is suggested to initiate PMN migration to an inflammatory site followed by CXCR1 mediated priming for anti-microbial activity.

IL-8 is perhaps best known for its proinflammatory effects on immune cells. In essence, IL-8 is secreted by multiple cell types exposed to inflammatory stimuli. For monocytes/macrophages, microbial exposure results in IL-8 release. This is followed by CXCR2-mediated chemotaxis of PMNs to sites of antigenic challenge, and their

subsequent activation/priming for anti-microbial activity. This activity is complemented by IL-8's ability to potentiate M-CSF action in the bone marrow that results in the release and maturation of granulocytes (14). IL-8 is also reported to have an angiogenic effect on tumor-associated endothelial cells. Here, tumor-derived IL-8 can act in a paracrine manner to activate CXCR1 and R2 on endothelial cells. Both receptors have been associated with PI3-K/Akt and RasGTP signaling, resulting in cell survival and proliferation. In addition, IL-8 positively regulates VEGF R2 and EGF R, two TKRs that mediate cell growth and migration (28).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-8 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 IL-8 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 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 Diluent 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)	166	414	708	164	410	751
Standard Deviation	6.3	18.3	33.2	9.4	23.4	33.4
CV%	3.8	4.4	4.7	5.7	5.7	4.4

B. RECOVERY

The recovery of human IL-8 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 96-118% with an average of 108%.

The recovery of human IL-8 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 78.7-87.7% with an average of 82.6%.

C. SENSITIVITY

The minimum detectable dose (MDD) of IL-8 is typically less than 7.8 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 highly purified *E.coli*-expressed recombinant human IL-8 produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of IL-8 and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	114	105 – 125
1:4	114	105 – 122
1:8	112	104 – 124
1:16	108	102 – 107

F. SAMPLE VALUES

Cell Culture Supernates -Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated for 3 days with 10 µg/mL PHA. An aliquot of the cell culture supernate was removed, assayed for levels of natural IL-8, and measured 403,000 pg/mL.

Serum - Four serum samples were evaluated for the presence of IL-8 in this assay. All samples measured ranged from 92.8 to 100.6 pg/mL with an average of 96.9 pg/mL.

G. SPECIFICITY

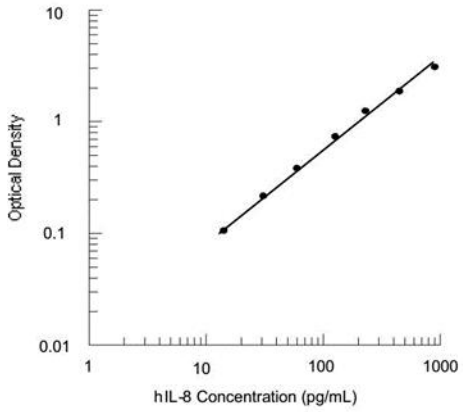
This assay recognizes both natural and recombinant human IL-8. The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhIL-8 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human	Recombinant mouse
GROα	KC
GROβ	MIP-1α
GROγ	MIP-1β
I-309	
IP-10	
MCP-1	
MCP-2	
MCP-3	
MIP-1α	
MIP-1β	

IV. EXPERIMENT

EXAMPLE STANDARD

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



pg/mL	OD	Average	Corrected
0	0.007 0.007	0.007	——
15.6	0.114 0.11	0.112	0.105
31.2	0.212 0.211	0.212	0.205
62.5	0.358 0.395	0.392	0.385
125	0.743 0.755	0.749	0.742
250	1.305 1.274	1.29	1.283
500	2.076 2.104	2.091	2.084
1000	3.065 2.994	3.03	3.023

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
IL-8 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IL-8	1 plate
IL-8 Conjugate	solution of polyclonal antibody against IL-8 conjugated to horseradish peroxidase	1 vial
IL-8 Standard	recombinant human IL-8 in a buffered protein base; lyophilized	1 vial
Calibrator Diluent(5×)	A 5× concentrated buffered protein base	1 vial
Wash Buffer Concentrate(25×)	A 25× concentrated solution of buffered surfactant	1 vial
Color Reagent A	Stabilized hydrogen peroxide	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine)	1 vial
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>Diluted Wash Solution</td><td rowspan="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Diluent 1×</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20°C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.</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.

D. PRECAUTION

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

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 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 x g. Remove serum and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

B. SAMPLE PREPARATION

Serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Diluent (1×).

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

Wash Solution - 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 Solution Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

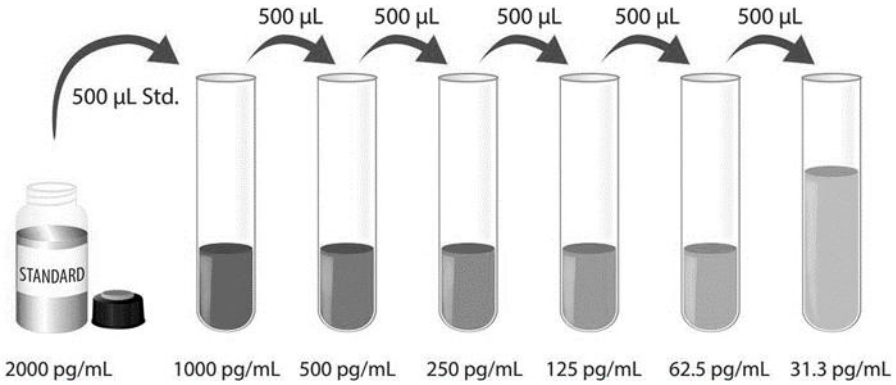
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Diluent 1× - Add 20 mL of Calibrator Diluent 5× into 80 mL of deionized or distilled water to prepare 100 mL of Diluent 1×.

IL-8 Standard - **Refer to the vial label for the reconstitution volume*.** This reconstitution produces a stock solution of 2000 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 500 µL of Diluent 1× into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The Diluent 1× 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.
- Substrate Solution 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 IL-8 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 plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Standard, sample, or control 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.
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 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.
5. Add 100 µL of IL-8 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
8. 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.
9. Determine the optical density of each well within 30 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.
10. **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 IL-8 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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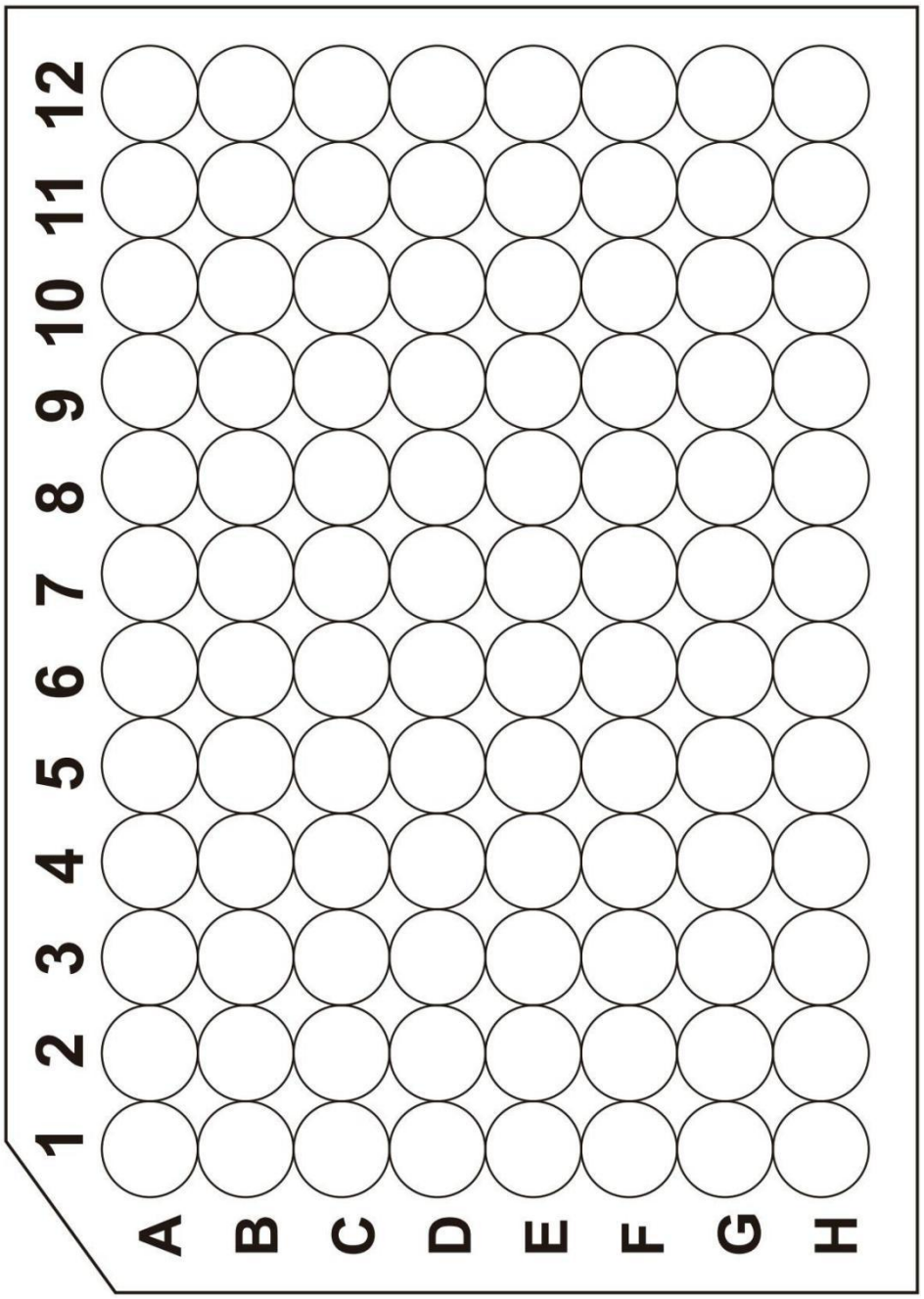
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 IL-8/CXCL8 Valukine™ ELISA 试剂盒

目录号: VAL103

适用于定量检测天然和重组人白介素 8（IL-8）的含量

科研专用，不可用于临床诊断

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Novus 试剂盒确保在你收货日期 3 个月内有效

目录

I. 背景

白细胞介素-8（IL-8，也称 GCP-1、NAP-1 和CXCL8）是属于α型或 CXC 家族的8-9kDa 的趋化因子，可与肝素结合。目前为止，共发现了 15 种人的 CXC 家族蛋白， 大小在 8-12 kDa 之间。绝大多数位于人类 4 号染色体，都具有典型的三β折叠层/一个α 螺旋结构，多数在 N 端显示 Glu-Leu-Arg 三肽基序（1-3）。人IL-8 先合成为一个 99 氨基酸前体，其中包含一个 20 氨基酸的信号序列，和一个 79 个氨基酸的成熟区域（4-6）。它可作为单体、同源二聚体、及与 CXCL4/PF4 形成的异源二聚体在体内循环。IL-8 单体被认为是最具有生物活性的，而异源二聚体可能会增强 PF4 的活性（7-10）。在氨基酸水平，成熟的人 IL-8 与猪和犬分别有65%和70%的同源性（11, 12）。在啮齿动物中，IL-8 的相应基因尚未发现。通过选择性剪接和差别蛋白水解产生了多种 IL-8 亚型。选择性剪接发生在 C-端，那里有一个 11 个氨基酸替代（位置在aa #92-99）（13）。蛋白酶水解作用可能是一个细胞的特定事件，它在IL-8 的 N-端产生截断。例如，成纤维细胞和血管内皮细胞将第 21 和第 22 氨基酸切断，形成 IL-8（1-7），而单体细胞和淋巴细胞在第 21-25 氨基酸切割，产生 IL-8（6, 7）。这些短式 IL-8 一般来说具有更高的生物活性，尤其是针对 CXCR1 IL-8 受体的活性增高（6, 14）。大约 15%的 IL-8 在前体Arg27 位发生了瓜氨酸化，这可提高 IL-8 的半衰期和促使白血球增多（15, 16）。很多类型的细胞都分泌IL-8，其中包括单核细胞和中性粒细胞（17）、成纤维细胞和角质形成细胞（18）、肥大细胞（19）、内脏平滑肌细胞（20）、树突状细胞（21）、II 型大肺泡细胞（22）和内皮细胞（23）。

IL-8 受体有两个，都属于G 蛋白耦联受体蛋白：CXCR1/IL-8RA 和 CXCR2/IL-8RB，二者之间的氨基酸序列享有 77%同源性（24）。CXCR1 分子量为 45-50kDa，几乎完全由 IL-8 独享；CXCR2 分子量为 35-40kDa，由所有的CXC 趋化因子所共有（25, 26）。

CXCR1 和 CXCR2 分别形成组成型同源二聚体，这似乎是它们的功能性构型。当同一个细胞表达时，异源二聚体也会形成；但当它与 IL-8 结合后便会被解体（27）。CXCR2 对低浓度 IL-8 有响应，且主要与趋化和 MMP-9 释放有关。与此相反，CXCR1 对高浓度 IL-8 有响应，并与呼吸爆发及磷脂酶 D2 的激活有关（26）。因此，在中性粒细胞CXCR2 被认为可引导中性粒细胞迁移到炎症部位，然后引发 CXCR1 介导的抗菌活性。

IL-8 最著名的功能是它在免疫细胞中的促炎作用。从本质上讲，IL-8 是由暴露于炎性刺激因子的多种细胞类型分泌。对于单核细胞/巨噬细胞来说，微生物的暴露引起 IL-8 的释放；随后，CXCR2 介导的趋化作用将中性粒细胞迁移到抗原攻击的部位，并伴随下一步抗菌活性的激活及启动。IL-8 可增强骨髓 M-CSF 的作用，从而引起粒细胞的成熟和释放（14）。IL-8 的这两个功能相辅相成。有报道说，IL-8 对肿瘤相关的内皮细胞具有血管生成功能。此时，肿瘤源性的 IL-8 可以采取一种旁分泌方式激活血管内皮细胞 的 CXCR1 和 CXCR2。CXCR1 和 CXCR2 都 与 PI3-K/Akt 和 RasGTP 信号通路相关， 参与细胞的存活和增殖。此外，IL-8 正调节 VEGFR2 和EGFR 属于介导细胞生长和迁移的受体络氨酸激酶（28）。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗人IL-8 单抗包被于微孔板上，样品/标准品中 的IL-8 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 IL-8 多抗，与结合在微孔板上的 IL-8 结合而形成免疫复合物，游离的成分被洗去； 加入底物溶液（显色剂），溶液颜色逐渐变成蓝色，加入终止液溶液变黄并且停止变化。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，应将样本用稀释剂（1×）稀释后重新检测； 若细胞培养上清液样本需分布稀释，除最后一步用稀释剂稀释外，其它中间稀释可采用细胞培养基；
- 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)	166	414	708	164	410	751
标准差	6.3	18.3	33.2	9.4	23.4	33.4
CV%	3.8	4.4	4.7	5.7	5.7	4.4

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IL-8，测定其回收率。回收率范围在 96-118%， 平均回收率在 108%。

在人血清样本中掺入检测范围内不同水平的人IL-8，测定其回收率。回收率范围在78.7-87.7%，平均回收率在82.6%。

C. 灵敏度

人 IL-8 的最低可测值一般小于 7.8 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 生产的大肠杆菌表达的高纯度重组人IL-8 蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人IL-8，然后用稀释剂（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	114	105 - 125
1:4	114	105 - 122
1:8	112	104 - 124
1:16	108	102 - 107

F. 样本预值

细胞上清样本 - 人的外周血单核细胞（1×10^6 细胞/mL）培养于含有10%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，100µg/mL 硫酸链霉素，另外 10 µg/mL PHA 刺激细胞，培养 3 天。取细胞培养上清液测定IL-8 含量，结果为 403,000 pg/mL。

血清样本 - 使用本试剂盒检测了4份人血清样本中IL-8 的水平。4份样本的检测值在92.8 - 100.6 pg/mL之间，平均值为96.9 pg/mL。

G. 特异性

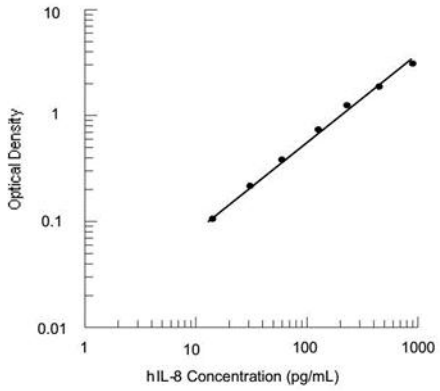
此 ELISA 法可检测天然及重组人 IL-8 蛋白。将以下因子用稀释剂（1×）配置成 50 ng/mL 的浓度来检测与人IL-8 的交叉反应。将50 ng/mL 的干扰因子掺入中间范围的重组人IL-8 对照品中，来检测对人 IL-8 的干扰。没有观察到明显的交叉反应或干扰。

重组人蛋白	重组小鼠蛋白
GROα	KC
GROβ	MIP-1α
GROγ	MIP-1β
I-309	
IP-10	
MCP-1	
MCP-2	
MCP-3	
MIP-1α	
MIP-1β	

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。



pg/mL	OD	Average	Corrected
0	0.007 0.007	0.007	——
15.6	0.114 0.11	0.112	0.105
31.2	0.212 0.211	0.212	0.205
62.5	0.358 0.395	0.392	0.385
125	0.743 0.755	0.749	0.742
250	1.305 1.274	1.29	1.283
500	2.076 2.104	2.091	2.084
1000	3.065 2.994	3.03	3.023

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
IL-8 Microplate	包被抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
IL-8 Conjugate	酶标IL-8 检测抗体	1 瓶
IL-8 Standard	标准品（冻干）	1 瓶
Calibrator Diluent（5×）	浓缩稀释剂（5×）	1 瓶
Wash Buffer Concentrate（25×）	浓缩洗涤缓冲液（25×）	1 瓶
Color Reagent A	显色液A	1 瓶
Color Reagent B	显色液B	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板胶纸	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="7">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="6">2-8℃储存，最多 30 天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>稀释剂（1×）</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>标准品</td><td>分装，-20℃储存最多 30 天*；避免反复冻融。</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
- 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

试剂盒中的终止液是酸性溶液，使用时请做好眼镜、手、面部及衣服的防护。

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。样本可能需要用稀释剂（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。

B. 样本准备工作

血清样本需要用稀释剂（1×）2倍稀释后进行检测，即100 µL血清+100 µL稀释剂（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温

注：*唾液中含有高浓度的IL-8，为避免污染，实验时请带口罩、手套。*

洗涤液：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 工作浓缩的洗涤液。未用完的放回 4℃。

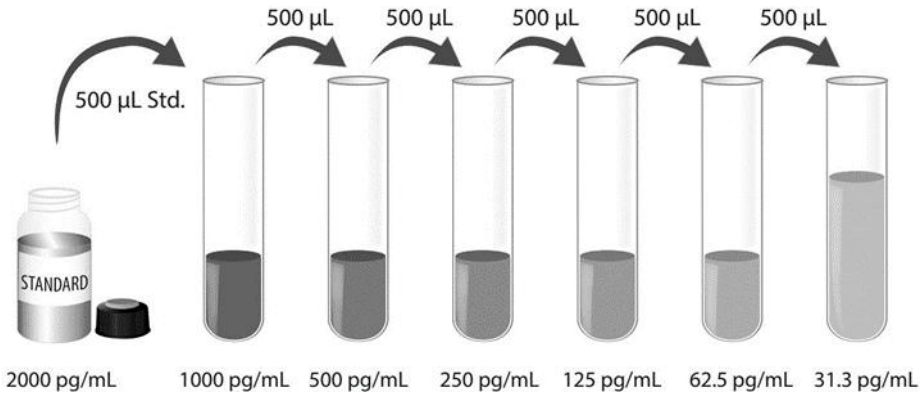
显色剂：按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合，避光；在使用前 15 分钟内准备，仅供当日使用；每孔需 200 µL。

稀释剂（1×）：可将 20 mL 浓缩稀释剂用 80 mL 蒸馏水或去离子水稀释配置成 100 mL 工作浓度的稀释剂。

标准品：重溶体积请参考瓶身标签重溶冻干标准品，得到浓度为 2000 pg/mL 标准品母液。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。

每个稀释管中加入 500 µL 稀释剂（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。1000 pg/mL 的标准品可用作标准曲线最高点，稀释剂（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，正确使用封板胶纸可保证结果的准确性；
- 混合后的显色底物在上板前应为无色，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显色底物上板顺序一致；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。用封板胶纸封住反应孔，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；
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 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 酶标检测抗体。用封板胶纸封住反应孔，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 200 µL 显色底物，室温孵育 30 分钟。**注意避光**；
8. 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；
9. 加入终止液后 30 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；
10. **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