PRODUCT INFORMATION & MANUAL

Human CCL11/Eotaxin Valukine™ ELISA

Catalog Number: VAL256

For the quantitative determination of natural and recombinant human Eotaxin concentrations

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

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

Version 202411.1

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I. BACKGROUND

Human Eotaxin is an 8.3 kDa, 74 amino acid (aa), non-glycosylated polypeptide that is a member of the CC family of chemokines (1, 2). Human Eotaxin exhibits considerable species cross-reactivity (3, 4), showing approximately 60% aa sequence identity with both mouse and guinea pig Eotaxins (2, 5, 6). In comparison with other human CC chemokines, Eotaxin shows 66% aa sequence identity with MCP-1 (1) and 58% aa sequence identity with MCP-4 (7). Eotaxin shows only 39% aa sequence identity with Eotaxin-2, a molecule named for its biological activity rather than for structural or sequence similarity to Eotaxin (8). Although Eotaxin may be constitutively produced (9), it appears most often to be induced by inflammatory cytokines such as IL-1, TNF-α and IFN-γ (5, 9-11). Cells reported to produce eotaxin are quite varied in type, including endothelial cells (2, 5, 12), fibroblasts (9), macrophages (2, 12), ciliated and non-ciliated bronchial epithelial cells (2, 12), smooth muscle cells (2, 12), chondrocytes (12), and eosinophils (2, 10).

Eotaxin has been shown to be a potent and selective chemoattractant for eosinophils during inflammation and allergic reactions (13). The activities of Eotaxin have been shown to be mediated by the human receptor CCR3, the third of eight currently known CC family G-protein coupled chemokine receptors (14-18). CCR3 is a serpentine 45-55 kDa, 355 aa protein that is characterized by the presence of seven transmembrane domains (19, 20). Although CCR3 is the only known receptor for Eotaxin (Kd = 100-500 pM), other chemokines do bind to CCR3, including Eotaxin-2 and MCP-4 (with high affinity), MCP-3, leukotactin-1, and RANTES (with lower affinity) (7, 8, 19, 21). Notably, modest changes in local pH and ionic strength markedly impact eotaxin binding to CCR3, suggesting that small perturbations of the tissue microenvironment may represent an additional mechanism for regulating the biological effects of Eotaxin (22). Cells known to express CCR3 include eosinophils (20), basophils (23, 24) and Type 2 T helper cells (Th2) (25).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Eotaxin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Eotaxin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Eotaxin 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 Eotaxin 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 and human plasma.
- 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 RD5K/RD6O and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

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

Inter-assay Precision (Precision between assays)

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

CELL CULTURE SUPERNATE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	66.8	184	390	67.5	193	396
Standard Deviation	2.0	8.4	23.5	7.8	18.2	40.2
CV%	3.0	4.6	6.0	11.6	9.4	10.2

HUMAN SERUM/PLASMA ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	64.5	109	377	63.4	190	400
Standard Deviation	2.5	5.8	12.8	7.3	19.6	33.5
CV%	3.9	5.3	3.4	11.5	10.3	8.4

B. RECOVERY

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

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=5)	100	86-111
Human Serum (n=5)	95	83-109
Human EDTA plasma (n=5)	99	82-115
Human Citrate plasma (n=5)	99	81-117

C. SENSITIVITY

The minimum detectable dose (MDD) of human Eotaxin is typically less than 5 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 *E. coli*-expressed recombinant human Eotaxin 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 Eotaxin and diluted with Calibrator Diluent RD5K/RD6O to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=5)	Human Serum (n=5)	Human EDTA plasma (n=5)	Human Citrate plasma (n=5)
1:2	Average % of Expected	101	105	102	106
	Range (%)	89-113	98-109	90-112	102-113
1:4	Average % of Expected	99	103	101	106
	Range (%)	96-104	94-113	91-110	98-117
1:8	Average % of Expected	97	103	104	106
	Range (%)	91-101	93-114	93-117	99-116
1:16	Average % of Expected	98	101	97	105
	Range (%)	93-103	92-112	89-115	98-116

F. SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human Serum (n=95)	163	50-642	78
Human EDTA plasma (n=34)	80	32-272	43
Human Citrate plasma (n=34)	132	47-399	81

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 × 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for human Eotaxin. All samples measured below the lowest Human Eotaxin Standard, 15.6 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human Eotaxin.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5K/RD6O and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Eotaxin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:
CCL1/I-309	CCL3/MIP-1α
CCL2/MCP-1	CCL4/MIP-1β
CCL3/MIP-1α	CXCL1/KC
CCL4/MIP-1β	
CCL5/RANTES	
CCL7/MCP-3	
CCL8/MCP-2	
CXCL1/GROα	
CXCL2/GROβ	
CXCL3/GROγ	
CXCL10/IP-10	

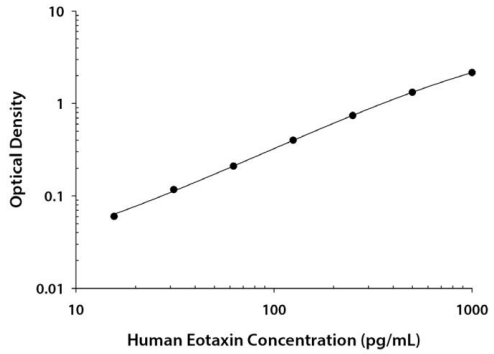
Recombinant mouse Eotaxin does not interfere but does cross-react approximately 0.03% in this assay.

IV. EXPERIMENT

EXAMPLE STANDARD

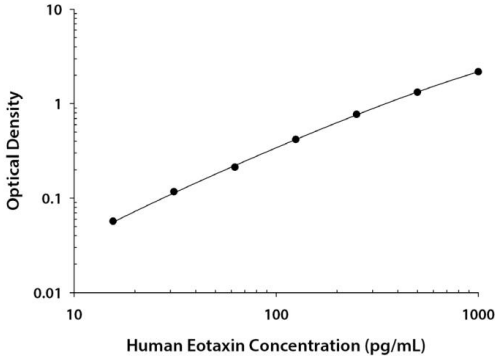
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.024 0.023	0.024	—
15.6	0.084 0.085	0.084	0.060
31.3	0.144 0.138	0.141	0.117
62.5	0.235 0.234	0.234	0.210
125	0.425 0.426	0.425	0.401
250	0.760 0.771	0.766	0.742
500	1.343 1.356	1.350	1.326
1000	2.219 2.144	2.182	2.158

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.029 0.029	0.029	—
15.6	0.084 0.089	0.086	0.057
31.3	0.148 0.144	0.146	0.117
62.5	0.239 0.246	0.242	0.213
125	0.444 0.447	0.446	0.417
250	0.778 0.817	0.798	0.769
500	1.347 1.362	1.354	1.325
1000	2.159 2.247	2.203	2.174

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Eotaxin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Eotaxin	1 plate
Human Eotaxin Conjugate	Solution of antibody against human Eotaxin conjugated to horseradish peroxidase	1 vial
Human Eotaxin Standard	Recombinant human Eotaxin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Assay Diluent RD1W	A buffered protein base	1 vial
Calibrator Diluent RD5K	A buffered protein base used to dilute standard and samples (*For cell culture supernate samples*)	1 vial
Calibrator Diluent RD6O	Animal serum used to dilute standard and samples (*For human serum/plasma samples. May contain a precipitate. Mix well before and during use.*)	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/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Discard the Eotaxin stock solution after 4 hours. Use a fresh standard for each assay.</td></tr>
<tr><td>Assay Diluent RD1W</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD5K</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD6O</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C.*</td></tr>
</table>

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

C. OTHER SUPPLIES REQUIRED

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

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.
- Calibrator Diluent RD6O contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 RD5K.

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 RD6O.

Plasma - Collect plasma using EDTA or citrate 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 RD6O.

Note: *Heparin plasma are not recommended for use in this assay.*

Hemolyzed samples are not suitable for use in this assay.

B. REAGENT PREPARATION

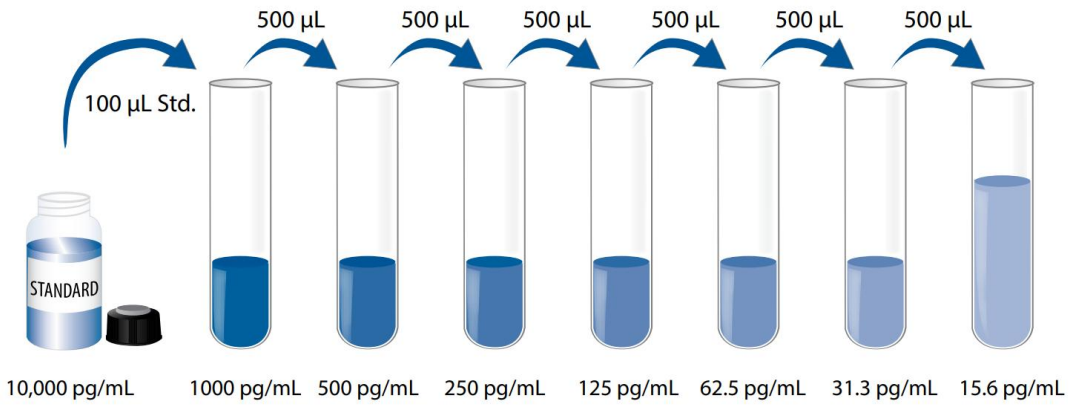
Bring all reagents to room temperature before use.

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 Eotaxin Standard - Refer to the vial label for the reconstitution volume*. Reconstitute the Human Eotaxin Standard with deionized or distilled water. This reconstitution produces a stock solution of 10000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

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

Pipette 900 µL of the Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6O (*for human serum/plasma samples*) into the 1000 pg/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



C. TECHNICAL HINTS

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

VII.ASSAY PROCEDURE

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

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 RD1W to each well.
4. Add 50 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process 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 Eotaxin 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. 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 Eotaxin 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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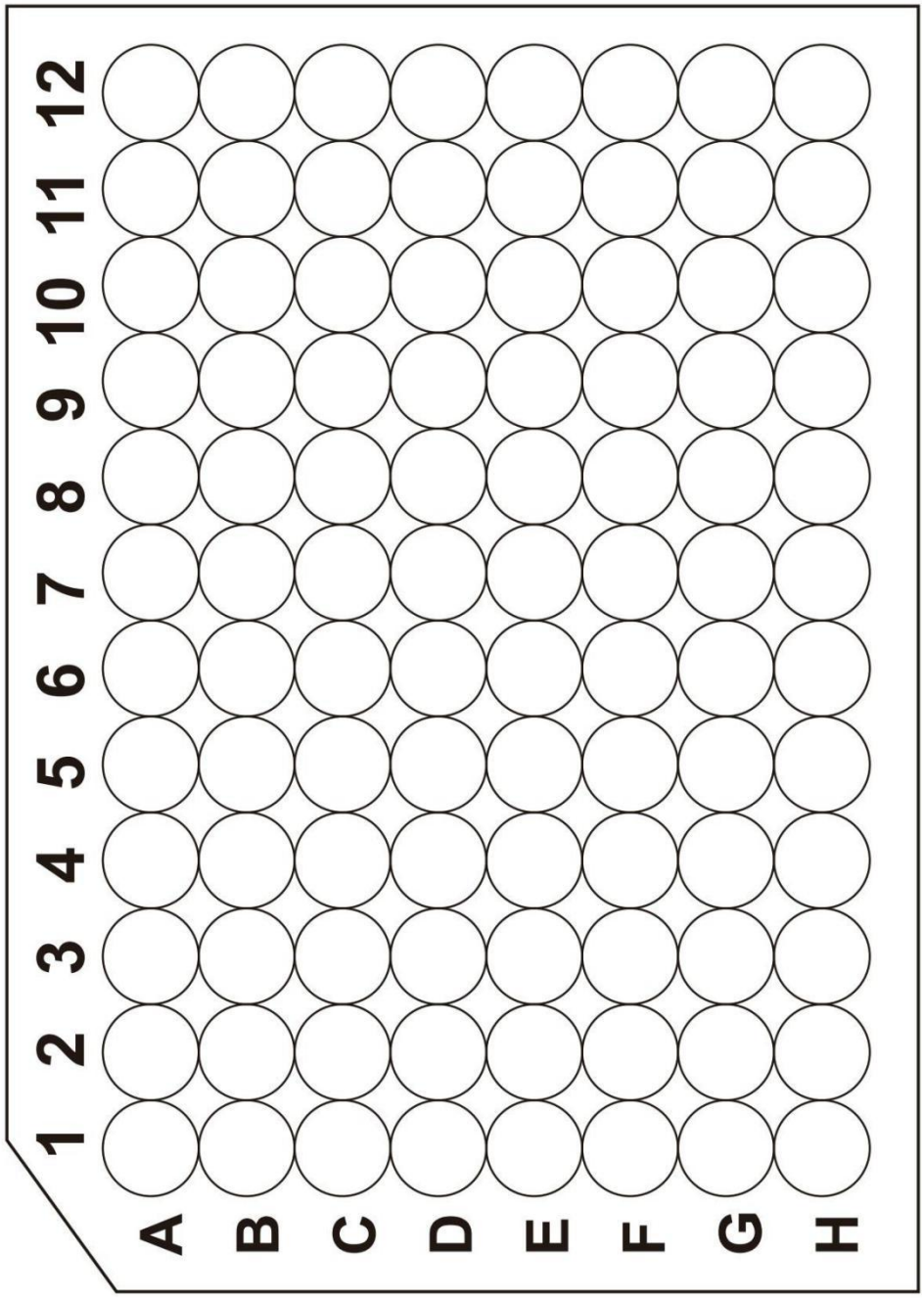
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 CCL11/Eotaxin Valukine™ ELISA 试剂盒

目录号：VAL256

适用于定量检测天然和重组人 Eotaxin 的浓度

科研专用，不可用于临床诊断

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版本号 202411.1

目录

I. 背景

人类 Eotaxin 是一种 8.3 kDa、74 氨基酸（aa）、非糖基化多肽，属于 CC 趋化因子家族（1, 2）。人类 Eotaxin 具有相当高的物种交叉反应性（3, 4），与小鼠和豚鼠的 Eotaxin 显示出约 60%的 aa 序列相同性（2, 5, 6）。与其他人类 CC 趋化因子相比，Eotaxin 与 MCP-1 的 aa 序列相同度为 66%（1），与 MCP-4 的 aa 序列相同度为 58%（7）。Eotaxin 与 Eotaxin-2 仅有 39%的 aa 序列相同，Eotaxin-2 是一种因其生物活性而命名的分子，而不是因其结构或序列与 Eotaxin 相似而命名（8）。虽然 Eotaxin 可能被组成性的产生的（9），但它似乎最常由 IL-1、TNF-α和 IFN-γ等炎症细胞因子诱导（5, 9-11）。据报道，可产生 eotaxin 的细胞类型多种多样，包括内皮细胞（2, 5, 12）、成纤维细胞（9）、巨噬细胞（2, 12）、纤毛和无纤毛支气管上皮细胞（2, 12）、平滑肌细胞（2, 12）、软骨细胞（12）以及嗜酸性粒细胞（2, 10）。

研究表明，在炎症和过敏反应期间，Eotaxin 对嗜酸性粒细胞具有强效的选择性趋化吸引作用（13）。研究表明，Eotaxin 的活性是由人类受体 CCR3 介导的，CCR3 是目前已知的八个 CC 家族 G 蛋白偶联趋化因子受体中的第三个（14-18）。CCR3 是一种 45-55 kDa、355 aa 的蛇形蛋白，其特点是存在七个跨膜结构域（19, 20）。虽然 CCR3 是唯一已知的 Eotaxin 受体（Kd = 100-500 pM），但其他趋化因子也会与 CCR3 结合，包括 Eotaxin-2 和 MCP-4（亲和力较高）、MCP-3、白细胞介素-1 和 RANTES（亲和力较低）（7, 8, 19, 21）。值得注意的是，局部 pH 值和离子强度的微小变化会明显影响 Eotaxin 与 CCR3 的结合，这表明组织微环境的微小扰动可能是调节 Eotaxin 生物效应的另一种机制（22）。已知表达 CCR3 的细胞包括嗜酸性粒细胞（20）、嗜碱性粒细胞（23, 24）和 2 型 T 辅助细胞（Th2）（25）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Eotaxin抗体包被于微孔板上，样品和标准品中的人Eotaxin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Eotaxin检测抗体进行孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清液，人血清和人血浆；
- 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RD5K/RD6O稀释并重复测定。
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测40次，以确定板间精确度。

细胞培养上清液测定

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（pg/mL）	66.8	184	390	67.5	193	396
标准差	2.0	8.4	23.5	7.8	18.2	40.2
CV%	3.0	4.6	6.0	11.6	9.4	10.2

人血清/血浆测定

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（pg/mL）	64.5	109	377	63.4	190	400
标准差	2.5	5.8	12.8	7.3	19.6	33.5
CV%	3.9	5.3	3.4	11.5	10.3	8.4

B. 回收率

不同类型样本中掺入检测范围内不同水平的人Eotaxin，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基（n=5）	100	86-111
人血清（n=5）	95	83-109
人 EDTA 血浆（n=5）	99	82-115
人柠檬酸盐血浆（n=5）	99	81-117

C. 灵敏度

人 Eotaxin 的最小可检测剂量（MDD）通常小于 5 pg/mL。

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

D. 校正

该免疫测定法以R&D Systems生产的高纯度的*E. coli*表达的重组人Eotaxin校正。

E. 线性

为评估测定的线性，不同的样本中含有或掺入高浓度的人Eotaxin，然后用标准品稀释液RD5K/RD6O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=5）	人血清（n=5）	人EDTA血浆（n=5）	人柠檬酸盐血浆（n=5）
1:2	平均值/期待值（%）	101	105	102	106
	范围（%）	89-113	98-109	90-112	102-113
1:4	平均值/期待值（%）	99	103	101	106
	范围（%）	96-104	94-113	91-110	98-117
1:8	平均值/期待值（%）	97	103	104	106
	范围（%）	91-101	93-114	93-117	99-116
1:16	平均值/期待值（%）	98	101	97	105
	范围（%）	93-103	92-112	89-115	98-116

F. 样本预值

人血清/血浆 - 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 Eotaxin。本研究中使用的供体没有病史。

样本类型	平均值（pg/mL）	范围（pg/mL）	标准差（pg/mL）
人血清（n=95）	163	50-642	78
人EDTA血浆（n=34）	80	32-272	43
人柠檬酸盐血浆（n=34）	132	47-399	81

细胞培养上清 - 人外周血单核细胞（5×10⁶ cells/mL）在补充了5%胎牛血清、50 μM β-巯基乙醇、2 mML-谷氨酰胺、100 units/mL青霉素和100 μg/mL链霉素硫酸盐的RPMI培养基中培养。细胞在不刺激或用10 μg/mL PHA刺激的情况下培养1天和5天。取培养上清液，测定天然人Eotaxin，所有样品测定值均低于最低人Eotaxin的标准品，15.6 pg/mL。

G. 特异性

该检测方法识别天然和重组人Eotaxin。

以下列出的蛋白在标准品稀释液RD5K/RD6O中以50 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的蛋白在中值范围重组人Eotaxin对照品中以50 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human:	**Recombinant mouse:**
CCL1/I-309	CCL3/MIP-1α
CCL2/MCP-1	CCL4/MIP-1β
CCL3/MIP-1α	CXCL1/KC
CCL4/MIP-1β	
CCL5/RANTES	
CCL7/MCP-3	
CCL8/MCP-2	
CXCL1/GROα	
CXCL2/GROβ	
CXCL3/GROγ	
CXCL10/IP-10	

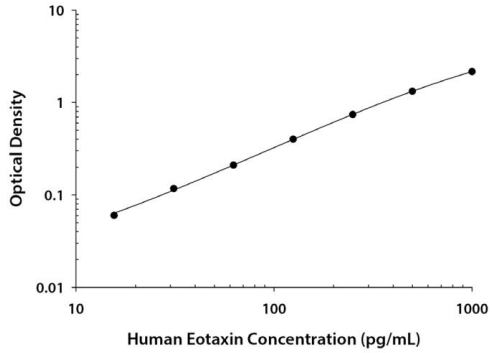
重组小鼠Eotaxin不会干扰，但在该检测中会发生约0.03%的交叉反应。

IV. 实验

标准曲线实例

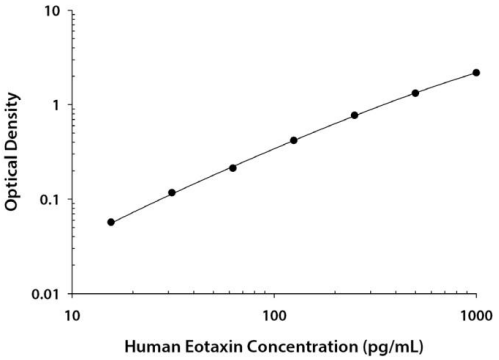
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.024 0.023	0.024	—
15.6	0.084 0.085	0.084	0.060
31.3	0.144 0.138	0.141	0.117
62.5	0.235 0.234	0.234	0.210
125	0.425 0.426	0.425	0.401
250	0.760 0.771	0.766	0.742
500	1.343 1.356	1.350	1.326
1000	2.219 2.144	2.182	2.158

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.029 0.029	0.029	—
15.6	0.084 0.089	0.086	0.057
31.3	0.148 0.144	0.146	0.117
62.5	0.239 0.246	0.242	0.213
125	0.444 0.447	0.446	0.417
250	0.778 0.817	0.798	0.769
500	1.347 1.362	1.354	1.325
1000	2.159 2.247	2.203	2.174

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Eotaxin Microplate	包被抗人Eotaxin抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human Eotaxin Conjugate	酶标检测抗人Eotaxin抗体	1瓶
Human Eotaxin Standard	重组人Eotaxin标准品（冻干），参考瓶身标签进行重溶	2瓶
Assay Diluent RD1W	检测液	1瓶
Calibrator Diluent RD5K	标准品稀释液，用于稀释标准品和样品 *（用于细胞培养上清液样品）*	1瓶
Calibrator Diluent RD6O	标准品稀释液，用于稀释标准品和样品 *（用于人血清/血浆样品。可能含有沉淀物。使用前和使用过程中请充分混合）*	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="9">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>Eotaxin储备溶液应在4小时后丢弃。每次测定都使用新的标准品。</td></tr>
<tr><td>检测液RD1W</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>标准品稀释液RD5K</td><td>2-8℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液RD6O</td><td>2-8℃储存，最多 30 天*</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
- 500 mL量筒
- 稀释标准品用的管子

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。
- 标准品稀释液RD6O含有叠氮化钠，该物质可能与铅和铜管发生反应，生成爆炸性的金属叠氮化物。处理时，请用大量清水冲洗。

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RD5K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RD6O稀释。

血浆 - 使EDTA或柠檬酸盐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RD6O稀释。

注：*本检测不建议使用肝素血浆。*

溶血样品不适用于本试验。

B. 检测前准备工作

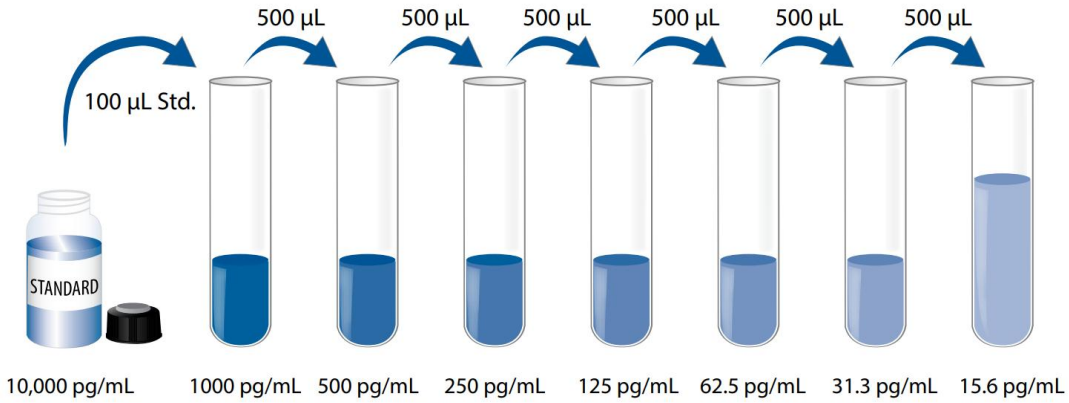
使用前请将所有试剂放置于室温。

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（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

人Eotaxin标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水复溶人Eotaxin标准品，得到浓度为10000 pg/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900 µL标准品稀释液RD5K（*用于细胞上清液样品*）或标准品稀释液RD6O（*用于人血清/血浆样品*）移入1000 pg/mL的管中，其余管中均加入500 µL相应的标准品稀释液。使用标准品储备母液制备以下稀释系列（如下图所示）。在每次转移之前，将每管彻底混合。1000 pg/mL作为标准曲线的最高标点。标准品稀释液作为标准曲线的零点（0 pg/mL）。



C. 技术小提示

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

VII.操作步骤

使用前，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

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. 向每个孔中加入100 µL的检测液RD1W。
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，**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Eotaxin酶标检测抗体。用新的封板膜封住反应孔，**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Eotaxin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