PRODUCT INFORMATION & MANUAL

Human CCL26/Eotaxin-3 Valukine™ ELISA

Catalog Number: VAL219

For the quantitative determination of natural and recombinant human Eotaxin-3 concentrations

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

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

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

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND

Eotaxin-3 (also known as CCL26 or SCYA26) is a CC chemokine with potent chemotactic activity for eosinophils (1-4). It was originally cloned from the GenBank human expressed sequence tag (EST) database (1). Eotaxin-3, along with Eotaxin-1 and Eotaxin-2, selectively activates the CC chemokine receptor 3 (CCR3). The Eotaxin-3-CCR3 interaction may play an important role in allergic diseases such as atopic dermatitis and bronchial asthma (5).

The full-length cDNA for Eotaxin-3 encodes a protein of 94 amino acids (aa) with a putative signal peptide of either 23 or 26 aa residues (1, 2). Both the 71 and 68 aa residue variants of recombinant Eotaxin-3 demonstrate equal potency in inducing chemotaxis of a human CCR3-transfected cell line (2). The mature Eotaxin-3 protein demonstrates 44% aa identity with MIP-1β and 40% aa identity with MIP-1α, RANTES and MCP-4 (1). Unlike most other CC chemokines, Eotaxin-3 maps to human chromosome 7q11.2, within 40 kilobases of the Eotaxin-2 loci (1, 2). Eotaxin-3 and Eotaxin-2 are unique in that they are the only chemokines identified to date that map to chromosome 7 (2, 6).

Like Eotaxin-1 and Eotaxin-2, Eotaxin-3 is a ligand for CCR3. The potency of Eotaxin-3 as a CCR3 ligand, however, is ten-fold less than that of Eotaxin-1 (2). CCR3 is a seven-transmembrane-spanning G-protein-linked receptor expressed on eosinophils, basophils, subpopulations of Th2 lymphocytes, and keratinocytes (5). Signal transduction via CCR3 is characterized by actin polymerization, a transient rise in cytosolic calcium concentration, and release of reactive oxygen species. Other chemokines capable of signaling through CCR3 include Eotaxin-1, Eotaxin-2, MCP-2, MCP-3, MCP-4, MIP-1δ, and RANTES. Studies indicate that Eotaxin-3 is capable of cross-desensitizing cells to other CCR3 ligands.

Northern blot analysis demonstrates that Eotaxin-3 mRNA is constitutively expressed within the heart, liver, and ovary (1, 2). Low levels of Eotaxin-3 expression can also be detected in a variety of other tissues (1). Expression of Eotaxin-3 mRNA in vascular endothelial cells can be up-regulated by the cytokines IL-13 and IL-4 in a STAT6-dependent fashion (3, 7). In contrast to other potent eosinophil chemoattractants (*i.e.* Eotaxin-1, RANTES and MCP-4) that are induced by proinflammatory cytokines, neither TNF-α, IL-1β, IFN-γ, nor TNF-α plus IFN-γ are effective at up-regulating Eotaxin-3 mRNA expression.

Eotaxin-3 appears to be important for contributing to eosinophil accumulation in atopic disease. The mRNA expression of Eotaxin-3, unlike Eotaxin-1 or Eotaxin-2, is up-regulated in asthmatics in the stages following allergen challenge (8). These data suggest the possibility that Eotaxin-3 may be responsible for the continuing recruitment of eosinophils to asthmatic airways during this period.

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Eotaxin-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Eotaxin-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Eotaxin-3 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-3 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, cell lysates, 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 (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 twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	43.6	86.0	131	44.4	91.7	139
Standard Deviation	0.570	1.06	1.77	3.66	7.79	11.6
CV%	1.3	1.2	1.4	8.2	8.5	8.3

B. RECOVERY

The recovery of human Eotaxin-3 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	92-119
Lysis buffer (n=1)	97	94-99
Human Serum (n=5)	93	81-106
Human EDTA Plasma (n=5)	94	84-105
Human Heparin Plasma (n=5)	95	86-109

C. SENSITIVITY

Twenty-six assays were evaluated and the minimum detectable dose (MDD) of human Eotaxin-3 ranged from 0.066-1.29 pg/mL. The mean MDD was 0.215 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-3 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-3 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates* (n=3)	Cell lysates* (n=3)	Human Serum (n=4)	Human EDTA plasma (n=4)	Human Heparin plasma (n=4)
1:2	Average % of Expected	97	99	92	93	101
	Range (%)	96-99	96-102	83-102	82-103	95-106
1:4	Average % of Expected	96	95	93	97	101
	Range (%)	93-99	91-100	82-103	85-106	95-111
1:8	Average % of Expected	96	93	90	100	91
	Range (%)	95-98	89-96	82-106	86-110	84-97
1:16	Average % of Expected	92	95	93	99	90
	Range (%)	91-93	93-96	81-107	88-110	80-100

*Samples were diluted prior to assay.

F. SAMPLE VALUES

Human Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Eotaxin-3 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human Serum (n=30)	8.87	93	ND-16.7
Human EDTA plasma (n=30)	7.35	93	ND-16.9

ND=Non-detectable

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Human Heparin plasma (n=30)	57.7	8.50-126	29.8

Note: *Human Eotaxin-3 interactions with epithelial cells and erythrocytes are disrupted by heparin, leading to the release of bound human Eotaxin-3 and increased sample values in Human Heparin plasma.*

Cell Culture Supernates - Human umbilical vein endothelial cells (HUVEC) (5×10^6 cells/mL) were cultured in EGM. Cells were cultured unstimulated or stimulated with 100 ng/mL of recombinant human IL-4 for 24 hours. Aliquots of the cell culture supernates were removed and assayed for human Eotaxin-3, and were non-detectable or measured 14,380 pg/mL, respectively.

Cell Lysates - HUVEC cells were cultured in EGM. Cells were cultured unstimulated or stimulated with 100 ng/mL of recombinant human IL-4 for 24 hours. Cells were then washed with PBS and solubilized in Lysis Buffer 17 using 3-5 times the pellet volume and put on ice for 15 minutes. Tubes were centrifuged at 14,000 × g for 5 minutes to remove insoluble material. The remaining whole cell extract was removed, aliquoted into a clean test tube, and stored at ≤ -70 °C. Whole cell extract protein concentration was quantified using a total protein assay 0.4 µg of the cell lysate was removed and

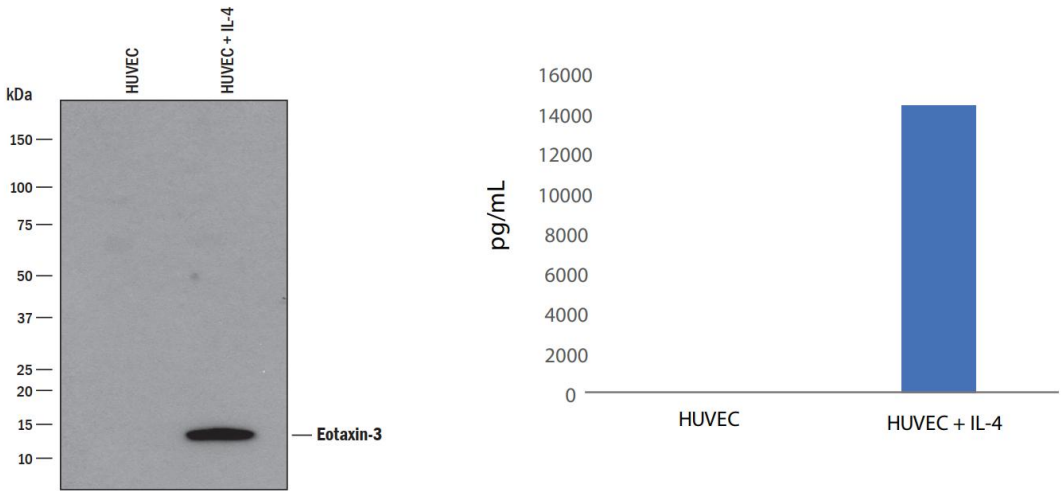
assayed for human Eotaxin-3. Unstimulated cell lysates were not detectable and stimulated lysates measured 89.9 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human Eotaxin-3.

The factors listed below were prepared at 2 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 2 ng/mL in a mid-range recombinant human Eotaxin-3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:		**Other recombinants:**
6CKine	MDC	mouse Eotaxin-3-like
CCL3L1	MIP-1α	rat Eotaxin-3-like
Eotaxin	MIP-1β	
Eotaxin-2	MIP-1δ	
I-309	MIP-3α	
IL-4	MIP-3β	
IL-13	RANTES	
MCP-1	Resistin	
MCP-2	STAT6	
MCP-3	TARC	
MCP-4	TECK	
	TRANCE	

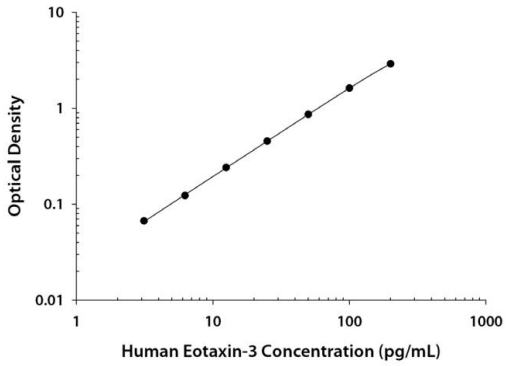


HUVEC cells were cultured in Endothelial Cell Growth Media (R&D Systems, Catalog # CCM027) until nearly confluent and then left untreated or treated with 100 ng/mL recombinant human IL-4 (R&D Systems, Catalog # 204-IL/CF) for 24 hours. The cell conditioned media was centrifuged to remove any cells or debris and then aliquoted. Cell samples were then analyzed by the Valukine Human Eotaxin-3 ELISA and Western Blot. For Western Blot, all samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with Gt x hCCL26/Eotaxin-3 (R&D Systems, Catalog # AF653). The Western Blot shows a direct correlation with ELISA sample values.

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)	O.D.	Average	Corrected
0	0.011 0.012	0.012	—
3.13	0.064 0.070	0.067	0.055
6.25	0.118 0.127	0.123	0.111
12.5	0.236 0.246	0.241	0.229
25	0.434 0.474	0.454	0.442
50	0.840 0.883	0.862	0.850
100	1.591 1.651	1.621	1.609
200	2.889 2.918	2.904	2.892

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Eotaxin-3 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Eotaxin-3	1 plate
Human Eotaxin-3 Conjugate	Solution of antibody against human Eotaxin-3 conjugated to horseradish peroxidase	1 vial
Human Eotaxin-3 Standard	Recombinant human Eotaxin-3 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Assay Diluent RD1S	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="6">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>Use a new standard for each assay.
Discard after use.</td></tr>
<tr><td>Assay Diluent RD1S</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent Concentrate (5×)/ RD5P</td><td>May be stored for up to 1 month at 2-8 °C.*
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
- Use polypropylene tubes for dilution of standards and samples.
- If using cell lysate samples, the following are also required:
  - Cell Lysis Buffer 17 (R&D Systems, Catalog # 895943)
  - PBS

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.
- Human Eotaxin-3 is detectable in human saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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×).

Cell Lysates - Cells must be lysed prior to assay as directed in the Sample Values section. 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.*

B. SAMPLE PREPARATION

Cell culture supernates and cell lysates may require dilution due to high endogenous levels. Multiple dilutions are recommended for unknown samples. Optimal dilutions should be determined by the end user.

For cell lysates, quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total cell lysate protein added is 0.1-0.4 µg/well. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Human Eotaxin-3 is found in human saliva. Wear a face mask and gloves to protect kit reagents from contamination.*

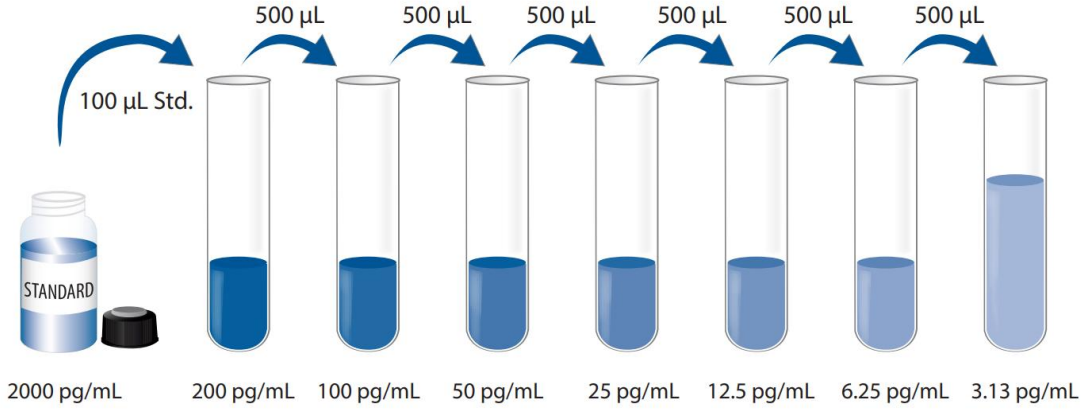
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Human Eotaxin-3 Standard - Refer to the vial label for the reconstitution volume* Reconstitute the Human Eotaxin-3 Standard with deionized or distilled water. 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.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent (1×) into the 200 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 pg/mL standard serves as the high standard. Calibrator 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.
- 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: *Human Eotaxin-3 is found in human saliva. Use of a face mask and gloves to protect kit reagents from contamination is recommended.*

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 RD1S to each well.
4. Add 100 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 three times for a total of four 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-3 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours 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 Eotaxin-3 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.

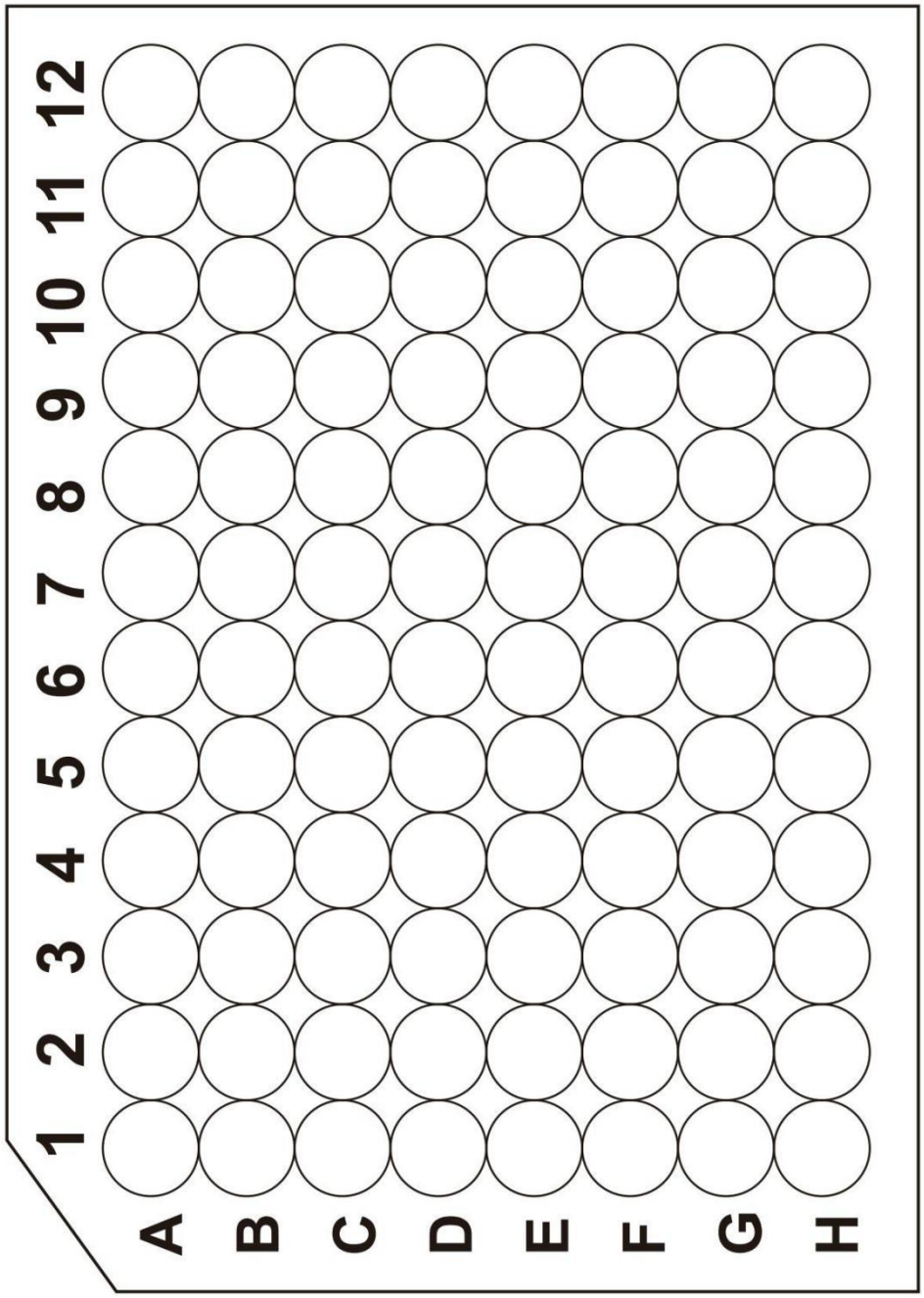
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Guo, R.F. et al. (1999) Genomics 58:313.
2. Kitaura, M. et al. (1999) J. Biol. Chem. 274:27975.
3. Shinkai, A. et al. (1999) J. Immunol. 163:1602.
4. Bandeira-Melo, C. et al. (2001) Am. J. Respir. Cell Mol. Biol. 24:653.
5. Petering, H. et al. (2001) J. Invest. Dermatol. 116:549.
6. Nomiyama, H. et al. (1998) Genomics 49:339.
7. Hoeck, J. and M. Woisetschlager (2001) J. Immunol. 167:3216.
8. Berkman, N. et al. (2001) Am. J. Respir. Cell Mol. Biol. 24:682.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 CCL26/Eotaxin-3 Valukine™ ELISA 试剂盒

目录号：VAL219

适用于定量检测天然和重组人 Eotaxin-3 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202411.1

目录

I. 背景

Eotaxin-3（又称 CCL26 或 SCYA26）是一种 CC 趋化因子，对嗜酸性粒细胞具有强大的趋化活性（1-4）它最初是从 GenBank 人类表达序列标签（EST）数据库中克隆出来的（1）。Eotaxin-3 以及 Eotaxin-1 和 Eotaxin-2 可选择性地激活 CC 趋化因子受体 3（CCR3）。Eotaxin-3-CCR3 相互作用可能在过敏性疾病（如特应性皮炎和支气管哮喘）中发挥重要作用（5）。

Eotaxin-3 的全长 cDNA 编码一个 94 个氨基酸（aa）的蛋白质，其中有一个 23 或 26 个 aa 残基的假定信号肽（1, 2）。重组 Eotaxin-3 的 71 aa 和 68 aa 残基变体在诱导人 CCR3 转染细胞系趋化方面具有同等效力（2）。成熟的 Eotaxin-3 蛋白与 MIP-1β有 44%的相同 aa，与 MIP-1α、RANTES 和 MCP-4 有 40%的相同 aa（1）。与大多数其他 CC 趋化因子不同，Eotaxin-3 映射到人类染色体 7q11.2，在 Eotaxin-2 loci 的 40 千碱基对范围内（1, 2）。Eotaxin-3 和 Eotaxin-2 的独特之处在于，它们是迄今为止发现的唯一映射到 7 号染色体上的趋化因子（2, 6）。

与 Eotaxin-1 和 Eotaxin-2 一样，Eotaxin-3 也是 CCR3 的配体。然而，Eotaxin-3 作为 CCR3 配体的效力比 Eotaxin-1 低十倍（2）。CCR3 是一种与 G 蛋白相连的七跨膜受体，在嗜酸性粒细胞、嗜碱性粒细胞、Th2 淋巴细胞亚群和角质形成细胞上都有表达（5）。通过 CCR3 进行信号转导的特点是肌动蛋白聚合、细胞膜钙浓度短暂升高以及释放活性氧。能够通过 CCR3 发出信号的其他趋化因子包括：Eotaxin-1、Eotaxin-2、MCP-2、MCP-3、MCP-4、MIP-1δ和 RANTES。研究表明，Eotaxin-3 能够使细胞对其他 CCR3 配体产生交叉脱敏作用。

Northern blot 分析表明，Eotaxin-3 mRNA 在心脏、肝脏和卵巢中呈组成型表达（1, 2）。在其他多种组织中也能检测到低水平的 Eotaxin-3 表达（1）。细胞因子 IL-13 和 IL-4 可通过 STAT6 依赖性方式上调血管内皮细胞中 Eotaxin-3 mRNA 的表达（3, 7）。与其他由促炎细胞因子诱导的强效嗜酸性粒细胞趋化诱导剂（即 Eotaxin-1、RANTES 和 MCP-4）相比，TNF-α、IL-1β、IFN-γ或 TNF-α加上 IFN-γ都不能有效上调 Eotaxin-3 mRNA 的表达。

Eotaxin-3 似乎是导致特应性疾病中嗜酸性粒细胞聚集的重要因素。与 Eotaxin-1 或 Eotaxin-2 不同，Eotaxin-3 的 mRNA 表达在过敏原挑战后的哮喘患者中上调（8）。这些数据表明，Eotaxin-3 可能是嗜酸性粒细胞在此期间持续进入哮喘气道的原因。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Eotaxin-3抗体包被于微孔板上，样品和标准品中的人Eotaxin-3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-3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，细胞裂解物，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（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）	43.6	86.0	131	44.4	91.7	139
标准差	0.570	1.06	1.77	3.66	7.79	11.6
CV%	1.3	1.2	1.4	8.2	8.5	8.3

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-3，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	92-119
裂解液（n=1）	97	94-99
人血清（n=5）	93	81-106
人 EDTA 血浆（n=5）	94	84-105
人肝素血浆（n=5）	95	86-109

C. 灵敏度

评估了 26 次测定，人 Eotaxin-3 的最小可检测剂量（MDD）范围为 0.066-1.29 pg/mL。平均 MDD 为 0.215 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生产的高纯度的大肠杆菌表达的重组人Eotaxin-3校正。

E. 线性

不同的样本中含有或掺入高浓度的人Eotaxin-3，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		细胞培养上清*（n=3）	细胞裂解物*（n=3）	人血清（n=4）	人EDTA血浆（n=4）	人肝素血浆（n=4）
1:2	平均值/期待值（%）	97	99	92	93	101
	范围（%）	96-99	96-102	83-102	82-103	95-106
1:4	平均值/期待值（%）	96	95	93	97	101
	范围（%）	93-99	91-100	82-103	85-106	95-111
1:8	平均值/期待值（%）	96	93	90	100	91
	范围（%）	95-98	89-96	82-106	86-110	84-97
1:16	平均值/期待值（%）	92	95	93	99	90
	范围（%）	91-93	93-96	81-107	88-110	80-100

*样本在检测前进行了稀释。

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-3。本研究中使用的供体没有病史。

样本类型	平均检测值（pg/mL）	%可检测范围	范围（pg/mL）
人血清（n=30）	8.87	93	ND-16.7
人EDTA血浆（n=30）	7.35	93	ND-16.9

ND=未检出

样本类型	平均值（pg/mL）	范围（pg/mL）	标准差（pg/mL）
人肝素血浆（n=30）	57.7	8.50-126	29.8

注：*肝素会干扰人Eotaxin-3与上皮细胞和红细胞的相互作用，导致结合的人 Eotaxin-3释放，并使人肝素血浆中的样本值升高。*

细胞培养上清 - 人脐静脉内皮细胞（HUVEC）（5 × 10^6 cells/mL）在EGM中进行培养。细胞未经刺激或用100 ng/mL的重组人IL-4刺激24小时。取细胞培养上清等分样本，检测人 Eotaxin-3，结果分别为未检出或测得14380 pg/mL。

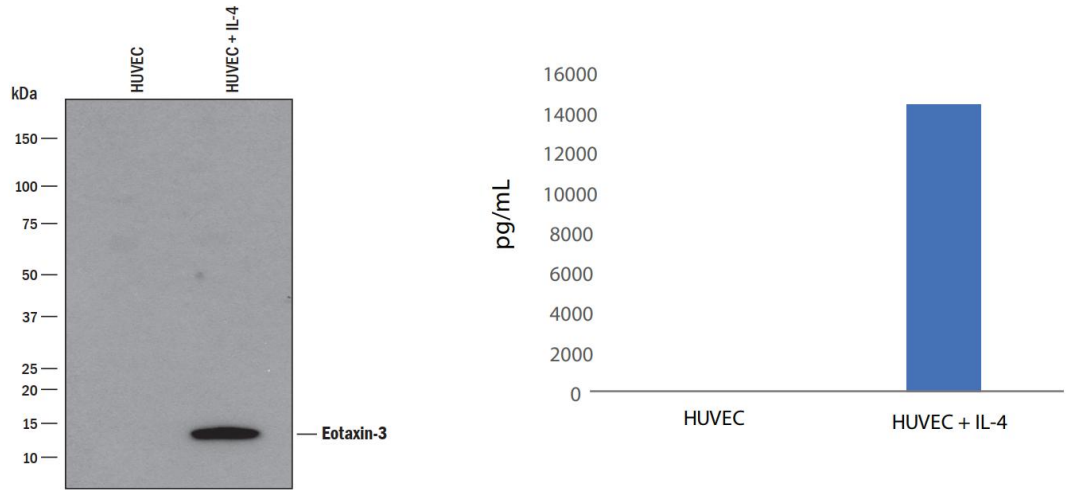
细胞裂解物 - HUVEC细胞在EGM培养基中培养。细胞未经刺激或用100 ng/mL重组人IL-4刺激培养24小时。随后用PBS洗涤细胞，用3-5倍沉淀体积的Lysis Buffer 17溶解，置于冰上15分钟。管子以14000 × g的转速离心5分钟以去除不溶性物质。将剩余的细胞提取物分装到干净管中，并储存在≤ -70 °C。使用总蛋白测定法测定细胞裂解物蛋白浓度，取0.4 µg的细胞裂解物进行人Eotaxin-3的测定。未经刺激的细胞裂解物未检出，刺激后的裂解物测量值为89.9 pg/mL。

G. 特异性

检测方法识别天然和重组人Eotaxin-3。

以下列出的因子在标准品稀释液（1×）中以2 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Eotaxin-3对照品中以2 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:		Other recombinants:
6CKine	MDC	mouse Eotaxin-3-like
CCL3L1	MIP-1α	rat Eotaxin-3-like
Eotaxin	MIP-1β	
Eotaxin-2	MIP-1δ	
I-309	MIP-3α	
IL-4	MIP-3β	
IL-13	RANTES	
MCP-1	Resistin	
MCP-2	STAT6	
MCP-3	TARC	
MCP-4	TECK	
	TRANCE	

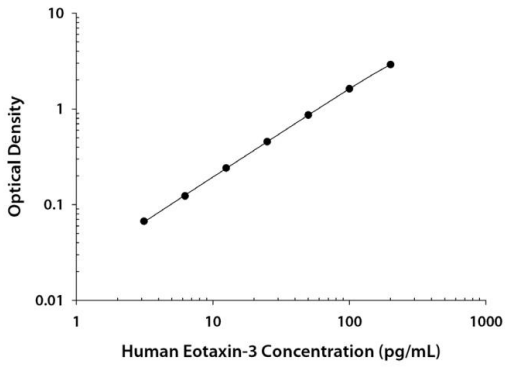


人脐静脉内皮细胞（HUVEC）在内皮细胞生长培养基（R&D Systems, 产品编号CCM027）中培养至近汇合，然后不进行处理或用100 ng/mL重组人IL-4（R&D Systems, 货号204-IL/CF）处理24小时。将细胞培养上清离心去除细胞和碎片，然后分装。然后通过Valukine Eotaxin-3 ELISA和Western印迹分析细胞样品。对于Western印迹，所有样品都在还原SDS-PAGE条件下分离，转移到PVDF膜上，并用Gt x hCCL26/ Eotaxin-3（R&D Systems, 货号AF653）进行免疫印迹。Western印迹结果与ELISA样本值直接相关。

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。



(pg/mL)	O.D.	Average	Corrected
0	0.011 0.012	0.012	—
3.13	0.064 0.070	0.067	0.055
6.25	0.118 0.127	0.123	0.111
12.5	0.236 0.246	0.241	0.229
25	0.434 0.474	0.454	0.442
50	0.840 0.883	0.862	0.850
100	1.591 1.651	1.621	1.609
200	2.889 2.918	2.904	2.892

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Eotaxin-3 Microplate	包被抗人Eotaxin-3抗体的96孔聚苯乙烯板，8孔×12条	1块板
Human Eotaxin-3 Conjugate	酶标检测抗人Eotaxin-3抗体	1瓶
Human Eotaxin-3 Standard	重组人Eotaxin-3标准品（冻干），参考瓶身标签进行重溶	2瓶
Assay Diluent RD1S	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="8">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>每次使用新的标准品，用后丢弃。</td></tr>
<tr><td>检测液RD1S</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°C 储存，最多 30 天*
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
- 使用聚丙烯管稀释标准品和样品
- 如果使用细胞裂解液样品，还需要满足以下条件：

  •细胞裂解液17（R&D Systems，货号895943）

  •PBS

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。
- 人Eotaxin-3可在人唾液中检测到。进行此测定时，请采取预防措施，防止试剂盒试剂污染。

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。

细胞裂解物 - 在进行检测之前，必须按照“样本预值”部分所述进行细胞裂解。样本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。*

B. 样品准备

细胞培养上清液和细胞裂解液由于内源性水平较高，可能需要稀释。建议对未知样品进行多次稀释。最佳稀释倍数应由最终用户确定。

对于细胞裂解液，建议使用总蛋白测定法定量样品蛋白浓度。建议加入的总细胞裂解液蛋白量为 0.1-0.4 µg/孔。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：*Eotaxin-3存在于唾液中。请佩戴口罩和手套以防止试剂盒试剂受污染。*

洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（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×）。

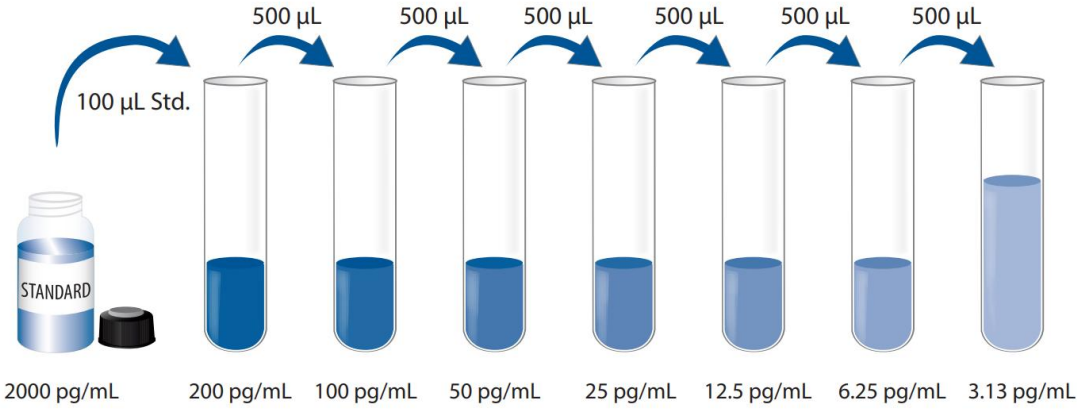
标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人Eotaxin-3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Eotaxin-3标准品，得到浓度为2000 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。

使用聚丙烯管。将900 µL标准品稀释液（1×）加入200 pg/mL的管中，剩余管中加入500

μL。使用标准品储备母液制备稀释系列（如下）。在进行下一次转移前，充分混匀每支管。200 pg/mL作为标准曲线最高点。标准品稀释液（1×）作为零点（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，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：*人Eotaxin-3存在于人唾液中。请佩戴口罩和手套以防止试剂盒试剂受污染。*

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RD1S。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，**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；
6. 在每个微孔内加入200 µL人Eotaxin-3酶标检测抗体。用新的封板膜封住反应孔，**用水平振荡器（0.12”轨道），转速：500 ± 50 rpm，室温孵育2小时**；
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-3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

1. Guo, R.F. et al. (1999) Genomics 58:313.
2. Kitaura, M. et al. (1999) J. Biol. Chem. 274:27975.
3. Shinkai, A. et al. (1999) J. Immunol. 163:1602.
4. Bandeira-Melo, C. et al. (2001) Am. J. Respir. Cell Mol. Biol. 24:653.
5. Petering, H. et al. (2001) J. Invest. Dermatol. 116:549.
6. Nomiyama, H. et al. (1998) Genomics 49:339.
7. Hoeck, J. and M. Woisetschlager (2001) J. Immunol. 167:3216.
8. Berkman, N. et al. (2001) Am. J. Respir. Cell Mol. Biol. 24:682.

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