PRODUCT INFORMATION & MANUAL

Mouse TARC/CCL17 Valukine™ ELISA

Catalog Number: VAL639

For the quantitative determination of natural and recombinant mouse Thymus and Activation-Regulated Chemokine (TARC) 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

Thymus and Activation-Regulated Chemokine (TARC/CCL17) is a member of the CC or β-chemokine family (1, 2). Mouse TARC cDNA encodes a highly basic 93 amino acid (aa) residue precursor protein with a 23 aa residue putative signal peptide that is cleaved to generate the 70 aa residue secreted protein. The mature protein is not glycosylated and has a predicted molecular weight of 8.0 kDa (1). Mouse TARC shares 64% and 83% aa sequence identity with human and rat TARC, respectively (3, 4). Among other β-chemokine family members, TARC is most closely related to MDC, sharing approximately 33% aa sequence homology (5). TARC mRNA is constitutively expressed at high levels in thymic dendritic cells and at lower levels in lymph node dendritic cells in the lung, colon and small intestine (1, 5). Additional cell types that have been shown to express TARC include keratinocytes (6), monocytes (7), $CD4^+$ T cells (8), fibroblasts (9), bronchial epithelial cells (10), and Reed-Sternberg cells (11).

The chemokine receptor CCR4 has been shown to be a high-affinity functional receptor for TARC (12). Mouse and human CCR4 share 85% aa sequence identity (13, 14). In humans, CCR4 expression can be detected on Th2 $CD4^+$ T cells (12, 15), basophils (13), IL-2 activated NK cells (16), and platelets (17). Although CCR8 has also been reported to bind TARC, this finding is controversial (18, 19).

Recombinant TARC has been shown to chemoattract T cell lines and CCR4-transfected cells (5). Mouse TARC has no chemotactic activity on naive peripheral $CD4^+$ T cells but is a chemoattractant for memory/effector $CD4^+$ T cells with a preference for Th2 cells (20). One of the principal functions of TARC may be to recruit effector/memory T helper cells to antigen presenting dendritic cells at sites of inflammation (20). TARC has been shown to selectively recruit skin-homing CLA^+ memory $CD4^+$ T cells into skin during times of immune challenge (21). TARC has been shown to chemoattract IL-2 activated NK cell (16) and to induce platelet aggregation and degranulation (17).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse TARC has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse TARC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse TARC 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 mouse TARC 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, mouse plasma and mouse 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 Calibrator Diluent RD6-12 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)	27	89	278	29	98	310
Standard Deviation	1.3	2.3	7.8	2.2	6.9	17.3
CV%	4.8	2.6	2.8	7.6	7.0	5.6

B. RECOVERY

The recovery of mouse TARC spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture supernates (n=6)	94	85-113
Mouse serum (n=6)	94	82-103
Mouse EDTA plasma (n=6)	94	83-109

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse TARC is typically less than 5.0 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 mouse TARC produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with high concentrations of mouse TARC in each matrix were diluted with Calibrator Diluent RD6-12 and assayed. Results from typical sample dilutions are shown.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Neat	311	____	____
	1:2	145	156	93%
	1:4	75	78	96%
	1:8	38	39	97%
	1:16	19	19	100%
Mouse serum	Spiked	367	____	____
	1:2	190	183	104%
	1:4	92	92	100%
	1:8	53	46	115%
	1:16	23	23	100%
Mouse EDTA plasma	Spiked	382	____	____
	1:2	202	191	106%
	1:4	106	96	110%
	1:8	49	48	102%
	1:16	25	24	104%

F. SAMPLE VALUES

Mouse serum/plasma - Samples were evaluated for detectable levels of mouse TARC in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum (n=20)	70	20-123	30
Mouse EDTA plasma (n=20)	69	32-100	20

Cell Culture Supernates：

Mouse splenocytes (1 x 10^6 cells/mL) were cultured for 3 days in RPMI plus 10% fetal bovine serum supplemented with 50 µM β-mercaptoethanol and 10 ng/mL recombinant human IL-2. An aliquot of the cell culture supernate was removed, assayed for mouse TARC, and measured 246 pg/mL.

Mouse lung conditioned media (2 lungs, 1-2 mm pieces in 40 mL of medium) were collected after culturing for 6 days in RPMI with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for mouse TARC, and measured 421 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant mouse TARC.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD6-12 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse TARC control were assayed for interference. No significant cross-reactivity or interference was observed.

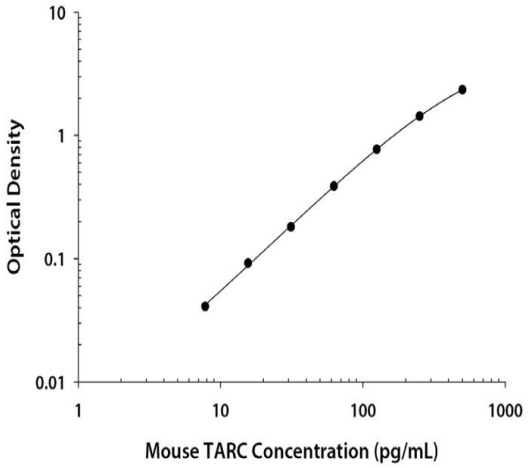
Recombinant mouse:			
C10	IL-3	IL-13	MIP-2
Eotaxin	IL-4	IL-17	OPG
Flt-3 Ligand	IL-5	IL-18	OSM
G-CSF	IL-6	JE/MCP-1	PlGF-2
GM-CSF	IL-7	KC	RANTES
IFN-γ	IL-9	LIF	SCF
IL-1α	IL-10	MARC	TNF-α
IL-1β	IL-10 R	MCP-5	TNF RI
IL-1ra	IL-12/IL-23 p40	M-CSF	TNF RII
IL-2	IL-12 p70	MIP-1α	Tpo
TRANCE	VEGF	VEGF R1	

Recombinant human TARC does not interfere but does cross-react approximately 0.1% in this assay.

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	0.051 0.054	0.052	—
7.8	0.094 0.092	0.093	0.041
15.6	0.144 0.145	0.144	0.092
31.3	0.235 0.231	0.233	0.181
62.5	0.435 0.445	0.440	0.388
125	0.807 0.839	0.823	0.771
250	1.458 1.504	1.481	1.429
500	2.271 2.527	2.399	2.347

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse TARC Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse TARC.	1 plate
Mouse TARC Conjugate	An antibody specific for mouse TARC conjugated to horseradish peroxidase.	1 vial
Mouse TARC Standard	Recombinant mouse TARC in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse TARC Control	Recombinant mouse TARC in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the vial label.	1 vial
Assay Diluent RD1W	A buffered protein base.	1 vial
Calibrator Diluent RD6-12	Diluted animal serum 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	Diluted hydrochloric 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>Wash Buffer (1×)</td><td rowspan="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Calibrator Diluent RD6-12</td></tr>
<tr><td>Control</td><td rowspan="2">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>Standard</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.
- Polypropylene test tubes for dilution of standards and samples.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6-12.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x 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 RD6-12.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000 x 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 RD6-12.

Note: *Heparin and citrate plasma have not been validated for use in this assay*.

Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

B. REAGENT PREPARATION

Bring all reagents to room temperature before use.

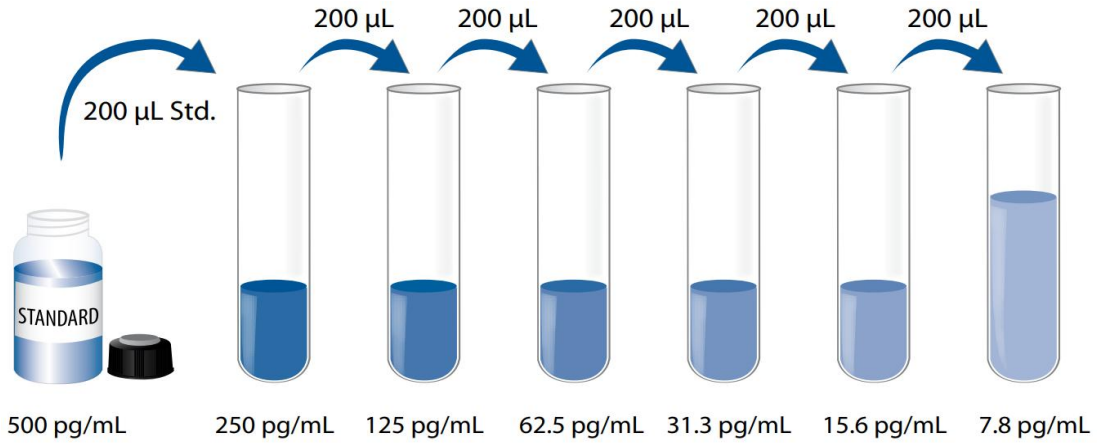
Mouse TARC Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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×).

Mouse TARC Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse TARC Standard with Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 500 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 200 µL of Calibrator Diluent RD6-12 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse TARC Standard (500 pg/mL) serves as the high standard. The Calibrator Diluent RD6-12 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 reagents and samples to room temperature before use. It is recommended that all samples, control and standards be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control and samples 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 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard, control and prepared sample per well. Mix by gently tapping the plate frame for 1 minute. 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 four times for a total of five 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 100 µL of Mouse TARC Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 100 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, control 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 mouse TARC 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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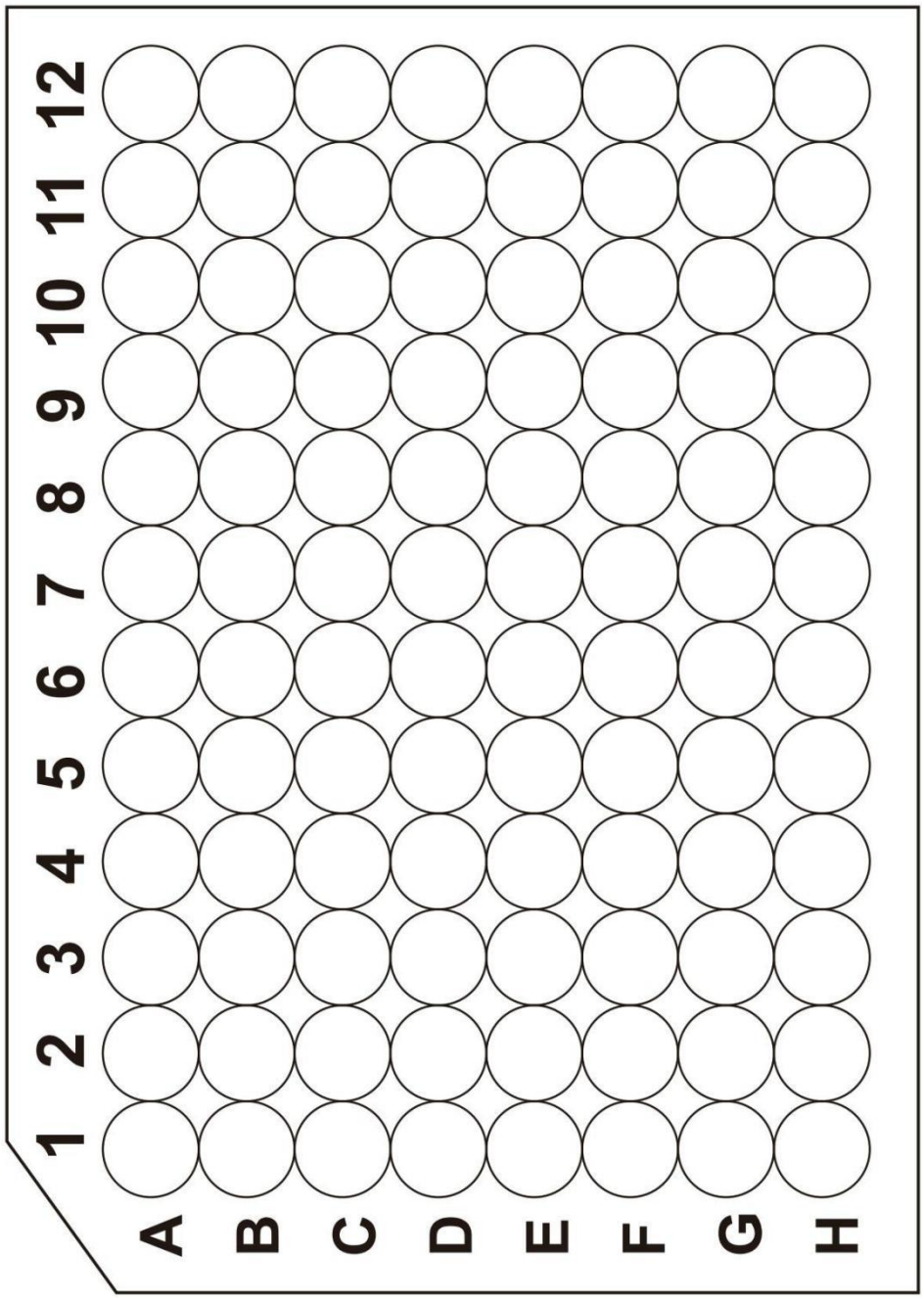
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 TARC/CCL17 Valukine™ ELISA 试剂盒

目录号：VAL639

适用于定量检测天然和重组小鼠胸腺和活化调节趋化因子（TARC）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

胸腺和激活调节趋化因子（TARC/CCL17）是 CC 或β-趋化因子家族的成员（1, 2）。小鼠 TARC cDNA 编码一种高碱性 93 氨基酸（aa）残基的前体蛋白，其中有 23 aa 残基的假定信号肽，信号肽被裂解后生成 70 aa 残基的分泌蛋白。成熟蛋白没有糖基化，预计分子量为 8.0 kDa（1）。小鼠 TARC 与人类和大鼠 TARC 分别有 64%和 83%的 aa 序列一致性。小鼠 TARC 与人类和大鼠 TARC 分别有 64%和 83%的 aa 序列一致性（3, 4）。在其他 β -趋化因子家族成员中，TARC 与 MDC 的关系最为密切，大约有 33%的 aa 序列同源性（5）。TARC mRNA 在胸腺树突状细胞中高水平持续表达，在肺、结肠和小肠的淋巴结树突状细胞中表达水平较低（1, 5）。已证明表达 TARC 的其他细胞类型包括角质形成细胞（6）、单核细胞（7）、$CD4^+$ T 细胞（8）、成纤维细胞（9）、支气管上皮细胞（10）和 Reed-Sternberg 细胞（11）。

趋化因子受体 CCR4 已被证明是 TARC 的高亲和力功能受体（12）。小鼠和人类的 CCR4 有 85%的 aa 序列一致性（13、14）。在人体内，Th2 $CD4^+$ T 细胞（12, 15）、嗜碱性粒细胞（13）、IL-2 激活的 N 细胞（16）和血小板（17）上都能检测到 CCR4 的表达。虽然也有报道称 CCR8 与 TARC 结合，但这一发现尚存争议（18, 19）。

重组 TARC 已被证明可趋化 T 细胞系和 CCR4 转染细胞（5）。小鼠 TARC 对幼稚外周 $CD4^+$ T 细胞无趋化活性，但对记忆/效应 $CD4^+$ T 细胞有趋化作用，并偏好 Th2 细胞（20）。TARC 的主要功能之一可能是将效应/记忆 T 辅助细胞招募到炎症部位的抗原呈递树突状细胞上（20）。有研究表明，TARC 能在免疫攻击期间选择性地将皮肤归巢的 CLA^+记忆 $CD4^+$ T 细胞招募到皮肤中（21）。已证明 TARC 可趋化吸引 IL-2 激活的 NK 细胞（16），并诱导血小板聚集和脱颗粒（17）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠TARC抗体包被于微孔板上。样品，质控品和标准品中的小鼠TARC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小鼠TARC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，小鼠血清样本和小鼠血浆样本；
- 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，应将样本用标准品稀释液RD6-12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)	27	89	278	29	98	310
标准差	1.3	2.3	7.8	2.2	6.9	17.3
CV%	4.8	2.6	2.8	7.6	7.0	5.6

B. 回收率

在不同类别样本中掺入检测范围内不同水平的小鼠TARC，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养上清 (n=6)	94	85-113
小鼠血清 (n=6)	94	82-103
小鼠EDTA血浆(n=6)	94	83-109

C. 灵敏度

小鼠TARC的最低可测剂量（MDD）一般小于5.0 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

该免疫检测法以R&D Systems生产的高纯度的大肠杆菌表达的重组小鼠TARC校正。

E. 线性

不同的样本类型（至少含有5个样本）中含有或掺入高浓度的小鼠TARC，然后用标准

品稀释液RD6-12将其稀释并测定其线性。下表所示为样本稀释的结果。

样本类型	稀释倍数	观测值 (pg/mL)	期望值 (pg/mL)	$\frac{\text{观测值}}{\text{期望值}}$ x 100
细胞培养上清样本	Neat	311	____	____
	1:2	145	156	93%
	1:4	75	78	96%
	1:8	38	39	97%
	1:16	19	19	100%
小鼠血清样本	Spiked	367	____	____
	1:2	190	183	104%
	1:4	92	92	100%
	1:8	53	46	115%
	1:16	23	23	100%
小鼠EDTA血浆样本	Spiked	382	____	____
	1:2	202	191	106%
	1:4	106	96	110%
	1:8	49	48	102%
	1:16	25	24	104%

F. 样本预值

小鼠血清/血浆样本 - 在此测定中对样本进行小鼠TARC的可检测水平的评估。

样本类型	平均值 (pg/mL)	范围(pg/mL)	标准差(pg/mL)
小鼠血清样本 (n=20)	70	20-123	30
小鼠EDTA血浆样本 (n=20)	69	32-100	20

细胞培养上清样本：

小鼠脾细胞（1 x 10^6 cells/mL）培养在含10%胎牛血清、50 μM β-巯基乙醇和10 ng/mL重组人 IL-2的RPMI培养基中，培养3 天。取出等分的细胞培养上清，检测小鼠TARC，结果为246 pg/mL。

小鼠肺组织（2个肺，1-2 mm块，放于40 mL培养基中）培养在含10%胎牛血清的RPMI条件培养基中，培养6天。取出等分的细胞培养上清，检测小鼠TARC，结果为421 pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠TARC。

将以下因子用标准品稀释液RD6-12配制成50 ng/mL的浓度来检测与小鼠TARC的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠TARC对照品中，来检测对小鼠TARC的干扰。没有观察到明显的交叉反应或干扰。

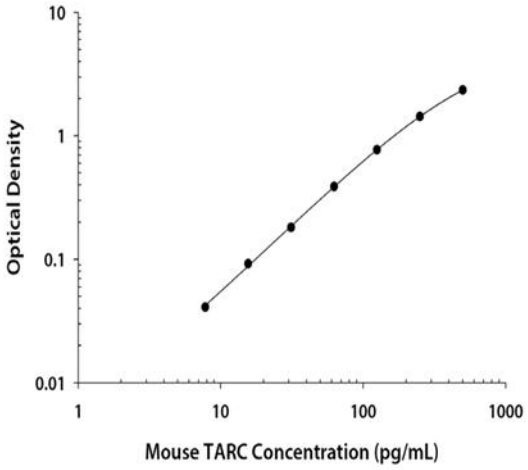
Recombinant mouse:			
C10	IL-3	IL-13	MIP-2
Eotaxin	IL-4	IL-17	OPG
Flt-3 Ligand	IL-5	IL-18	OSM
G-CSF	IL-6	JE/MCP-1	PlGF-2
GM-CSF	IL-7	KC	RANTES
IFN-γ	IL-9	LIF	SCF
IL-1α	IL-10	MARC	TNF-α
IL-1β	IL-10 R	MCP-5	TNF RI
IL-1ra	IL-12/IL-23 p40	M-CSF	TNF RII
IL-2	IL-12 p70	MIP-1α	Tpo
TRANCE	VEGF	VEGF R1	

重组人TARC在本试验中没有干扰，但会有约0.1%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。



(pg/mL)	O.D.	Average	Corrected
0	0.051 0.054	0.052	—
7.8	0.094 0.092	0.093	0.041
15.6	0.144 0.145	0.144	0.092
31.3	0.235 0.231	0.233	0.181
62.5	0.435 0.445	0.440	0.388
125	0.807 0.839	0.823	0.771
250	1.458 1.504	1.481	1.429
500	2.271 2.527	2.399	2.347

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse TARC Microplate	包被抗小鼠TARC抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse TARC Conjugate	酶标检测抗小鼠TARC抗体	1瓶
Mouse TARC Standard	小鼠TARC标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse TARC Control	小鼠TARC质控品（冻干），质控品的测定值应在标签上规定的范围内	1瓶
Assay Diluent RD1W	检测液	1瓶
Calibrator Diluent RD6-12	标准品稀释液用于稀释标准品和样本	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℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1W</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>标准品稀释液RD6-12</td></tr>
<tr><td>质控品</td><td rowspan="2">分装并≤ -20°C 储存，最多 30 天*，避免反复冻融。</td></tr>
<tr><td>标准品</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450nm检测波长的吸收值及540nm或570nm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
- 500mL量筒
- 用于稀释标准品和样品的聚丙烯管

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样本收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样本稳定性尚未评估。

细胞培养上清：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液RD6-12稀释。

血清样本：血液样本在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用标准品稀释液RD6-12稀释。

血浆样本：使用 EDTA作为抗凝剂收集血浆。然后2000 × g离心20分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤ -20°C储存备用。避免反复冻融。样本可能需要用稀释液RD6-12稀释。

注意： *本试剂盒对枸橼酸钠血浆和肝素血浆尚未被验证。*

本试剂盒不适合用于溶血或血脂过高样本。

B. 检测前准备工作

使用前请将所有试剂放置于室温。

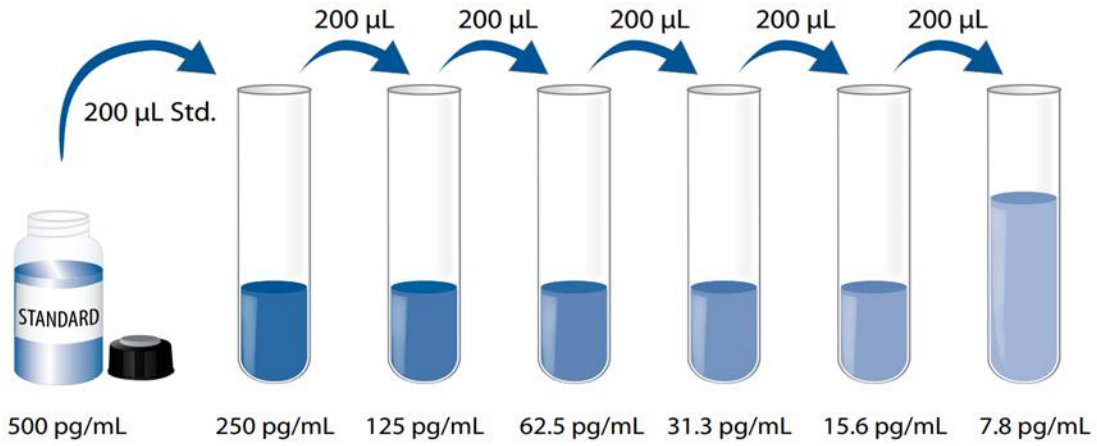
小鼠TARC质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

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×）。

小鼠TARC标准品：重溶体积请参考瓶身标签*，用标准品稀释液RD6-12重溶小鼠TARC标准品，请勿使用其他稀释液。得到浓度为500 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。

使用聚丙烯管，向每一稀释管中加入200 µL标准品稀释液RD6-12。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。未稀释的小鼠TARC标准品可用作标准曲线最高点（500 pg/mL），标准品稀释液RD6-12可用作标准曲线零点（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

使用前请将所有试剂和样本放置于室温。建议所有的实验样本，质控品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，标准品，质控品和样本；
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. 每孔加入50μL检测液RD1W。
4. 分别将不同浓度标准品，质控品和实验样本加入相应孔中，每孔50 μL。轻轻拍打微孔板1分钟，后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4次，共洗5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100 μL小鼠TARC酶标检测抗体。用封板膜封住反应孔，**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100 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100 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，质控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。数据可以通过绘制小鼠TARC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