PRODUCT INFORMATION & MANUAL

Mouse IL-17 Valukine™ ELISA

VAL610

For the quantitative determination of natural and recombinant mouse Interleukin IL-17 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

Mouse Interleukin 17 (IL-17; also IL-17A and CTLA-8) is a 21 kDa, variably glycosylated polypeptide that belongs to the IL-17 family of cytokines containing a cysteine-knot fold (1-3). Its sequence was originally isolated from an activated hybridoma created from the fusion of a mouse cytotoxic and rat T cell lymphoma cell line (2-5). It is synthesized as a 158 amino acid (aa) precursor that contains a 25 aa signal sequence and a 15 kDa, 133 aa mature segment (5). In both mouse and human, there is one conserved N-linked glycosylation site that likely contributes 5 kDa to its native molecular weight. IL-17A forms both a 35-38 kDa homodimer, and a 45-48 kDa heterodimer with IL-17F (6, 7). Mature mouse IL-17A is 61% and 89% aa identical to human and rat IL-17A, respectively (4, 5, 8). While rodent and human mature sequences show modest aa sequence identity, human IL-17 is active on both mouse and rat cells (5, 9). Cells known to produce IL-17 are the $CD4^+$ Th17 T cells, Paneth cells, $GR1^+CD11b^+$ myeloid suppressor cells, CD27-γδ T cells, $CD1^+NK1.1^-$ iNKT cells and $CD3^-CD4^+$ LTi-like cells (3, 5, 6, 10-12).

A high affinity receptor for mouse IL-17 has been reported, and appears to be a heteromultimer of IL-17RA and IL-17RC, likely in a 2:1 ratio (1). IL-17RA is a 130 kDa, type I transmembrane glycoprotein that bears no resemblance to members of the cytokine, TNF or immunoglobulin receptor superfamily (2, 10, 13). IL-17RC is also a type I transmembrane protein, approximately 90-95 kDa in size, that shares less than 30% aa identity with IL-17RA (14, 15). Both receptors are needed for IL-17A and IL-17A: F activity. The two receptors appear to form a functional association following ligand binding to IL-17RA (1, 16).

IL-17 is best known for its participation in the recruitment and survival of neutrophils (3, 10, 17, 18). Its induction was initially described to be the result of antigen stimulation of DC, resulting in IL-23 secretion. In a TCR-independent event, IL-23 induces T cell production of IL-17 (3). Once secreted, IL-17, in the bone marrow would seem to induce stromal/fibroblast expression of both G-CSF and stem cell factor (membrane form), an effect that increases PMN differentiation and production. IL-17 may complement this by directly blocking neutrophil apoptosis, promoting greater circulating PMN numbers (17). In the tissues, IL-17 would also seem to promote neutrophil extravasation, principally through its effects on macrophages and endothelial cells (EC). On macrophages, IL-17 induces TNF-α, IL-1β and IL-6 production (19). TNF-α and IL-1β then act on local ECs to induce G-CSF secretion, an effect that is potentiated by IL-17 (20). IL-17 further contributes to PMN influx by inducing EC CXC chemokine release and NO production, which may increase vascular permeability (3, 9). IL-17 effects are not limited to neutrophils. In synovial joints, IL-17 upregulates RANKL expression on osteoblasts. This provides a stimulus for osteoclast formation and subsequent bone resorption (18).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-17 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-17 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-17 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 mouse IL-17 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 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

Two 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	1	2	3
n	20	20	20	20	20
Mean (pg/mL)	40.6	432	41.2	51.9	480
Standard Deviation	3.5	26.2	3.7	4.6	40.9
CV%	8.6	6.1	9.0	8.9	8.5

B. RECOVERY

The recovery of mouse IL-17 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 82-110% with an average of 94%.

The recovery of mouse IL-17 spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 79.5-92.0% with an average of 86.1%。

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-17 is typically less than 1.8 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 mouse IL-17 produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of mouse IL-17 and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	107	100 - 111
1:4	109	104 - 119
1:8	107	95 - 120
1:16	102	88 - 120

F. SAMPLE VALUES

Cell Culture Supernates - Two spleen organ tissues from a mouse were homogenized and seeded in 100 mL of RPMI1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine,100 U/mL penicillin,100 µg/mL streptomycin sulfate, and 10 µg/mL Con A for 2 days. The cell culture supernate was assayed for mouse IL-17 and measured 3424 pg/mL.

EL-4 cells (Mouse thymoma) were seeded at 2×10^5 cells/mL and cultured for 4 days in 100 mL of DMEM supplemented with 10% horse serum, 10 g/mL PHA and 10 ng/mL PMA. The cell culture supernate was assayed for mouse IL-17 and measured 17 pg/mL.

Serum - Four Serum samples were evaluated for the presence of IL-17 in this assay. All samples measured ranged from 33.5 to 46.0 pg/mL with an average of 38.4 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse IL-17. 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 recombinant mouse IL-17 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Mouse

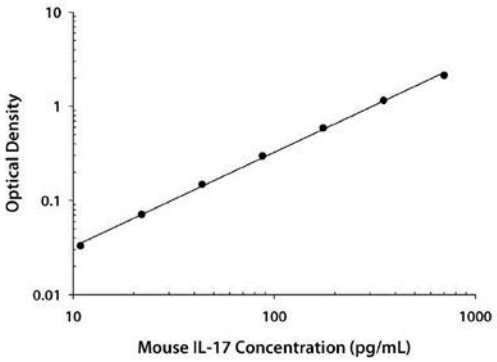
IL-17 B (aa 1-180)	IL-17 C
IL-17 B (aa 21-180)	IL-17 D
IL-17 BR	IL-17 E
IL-17 RC	IL-17 F
IL-17 RD	

There is 66% cross-reactivity observed with recombinant mouse IL-17 A/F Heterodimer. At concentration 10 ng/mL or greater, mouse IL-17R interferes in this assay. At concentrations greater than 78 pg/mL, rmIL-17 R1 interferes 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	0.032 0.032	0.032	—
10.9	0.064 0.066	0.065	0.033
21.9	0.103 0.103	0.103	0.071
43.8	0.177 0.183	0.180	0.148
87.5	0.321 0.339	0.330	0.298
175	0.604 0.634	0.619	0.587
350	1.158 1.213	1.186	1.154
700	2.123 2.204	2.164	2.132

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IL-17 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody against mouse IL-17	1 plate
Mouse IL-17 Conjugate	Solution of a polyclonal antibody against mouse IL-17 conjugated to horseradish peroxidase	1 vial
Mouse IL-17 Standard	Recombinant mouse IL-17 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	Diluted hydrochloric acid solution	1 vial
Plate Covers	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.

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 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator 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 Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

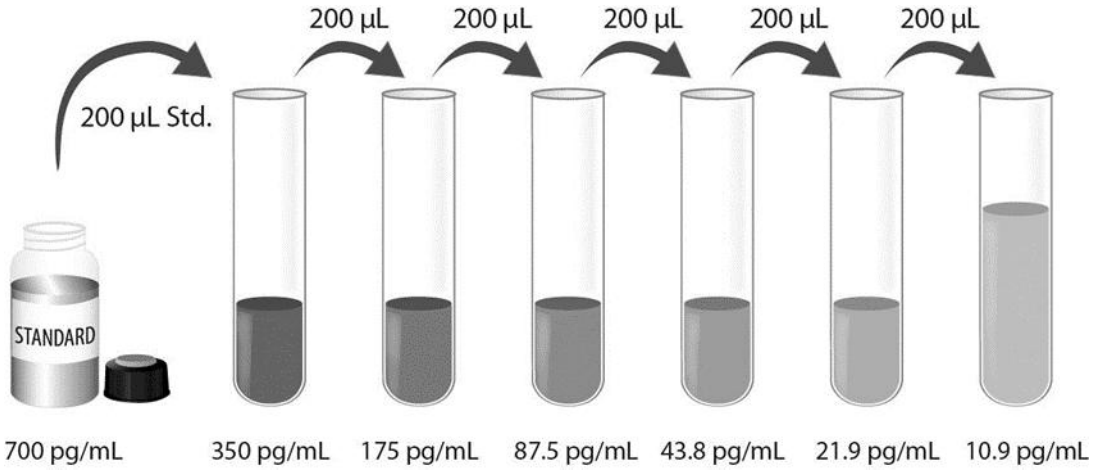
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

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

Mouse IL-17 Standard - Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 700 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

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

Pipette 200 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 undiluted standard 700 pg/mL 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

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Diluent 1× to each well.
4. Add 50 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.
5. 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.
6. Add 100 µL of mouse IL-17 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 Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
9. Add 100 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.
10. 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.
11. **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-17 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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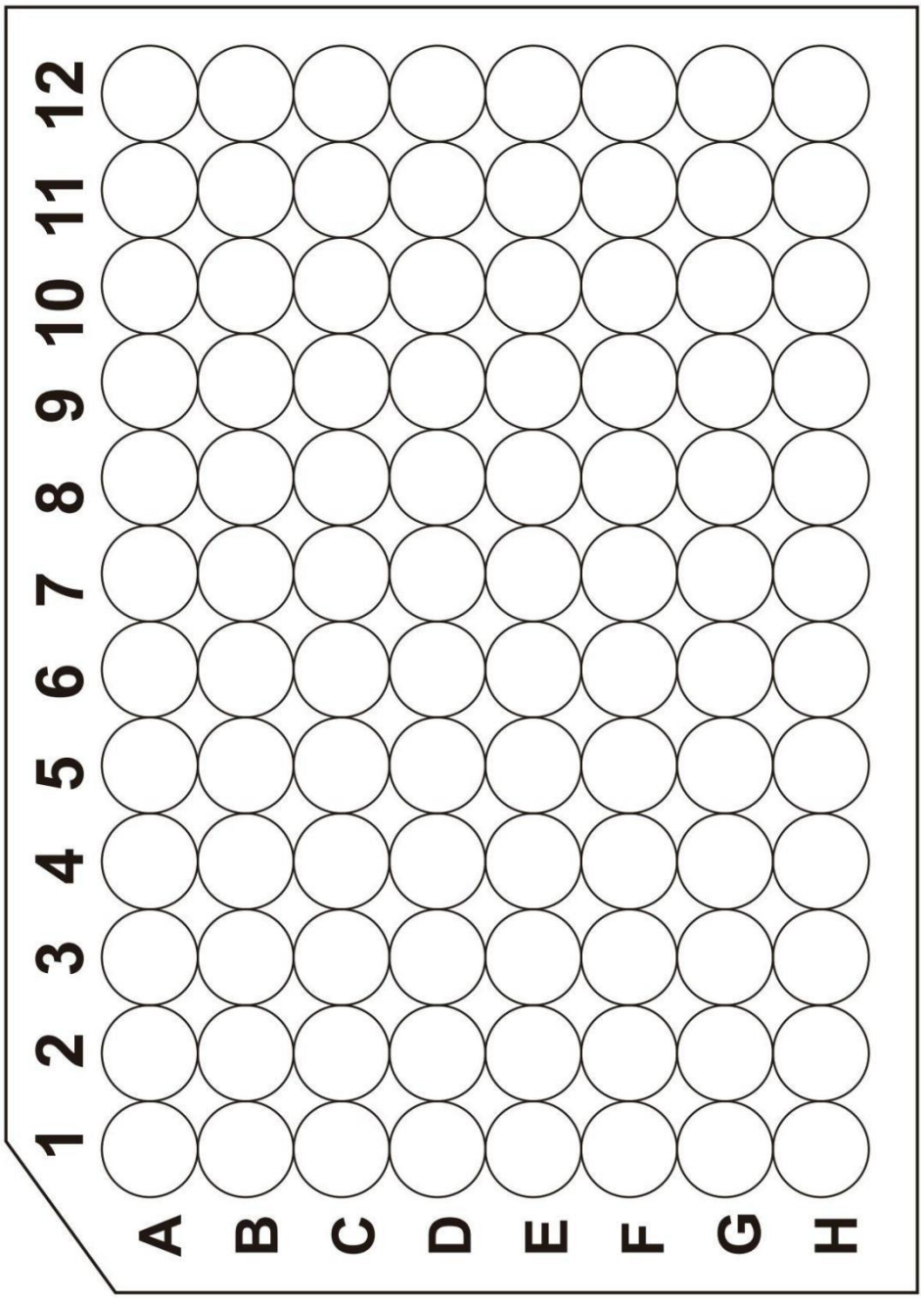
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠IL-17 Valukine™ ELISA 试剂盒

目录号：VAL610

适用于定量检测天然和重组小鼠IL-17的浓度

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

小鼠白细胞介素 17（IL-17，也叫IL-17A,或 CTLA-8）, 是一个 21 kDa 的不同程度糖基化的多肽，属于 IL-17 家族。该家族的细胞因子均含有一个半胱氨酸结的折叠（1-3）。人们最初是从小鼠的细胞毒性细胞和大鼠的 T 淋巴细胞融合而成的被激活的杂交瘤细胞中分离得到的 IL-17（2-5）。小鼠 IL-17 前体由 158 个氨基酸组成，包括一个 25 个氨基酸的信号肽和一个 15kDa 的133 个氨基酸的成熟肽（5）。人和小鼠的IL-17 都有一个保守的N-端糖基化位点，该位点约 5kDa。IL-17A 可形成一个 35-38kDa 的同源二聚体或与 IL-17F 形成一个 45-48 的异源二聚体（6-7）。成熟的小鼠IL-17A 与人和大鼠的IL-17A 氨基酸同源性分别为 61%和89%（4,5,8）。尽管啮齿类动物和人的成熟 IL-17A 序列仅有一定程度的同源性，但人的IL-17 对小鼠和大鼠的细胞也有活性 (5-9) 。分泌IL-17 的细胞有$CD4^+$ Th17 T 细胞、帕内特细胞、$GR1^+CD11b^+$ 髓抑制细胞、$CD27^-$γδ T 细胞、$CD1^+$ $NK1.1^-$iNK T 细胞和$CD3^-CD4^+$ LTi 一样细胞（3, 5, 6, 10-12）。

小鼠 IL-17 的高亲和受体已有报道，是一种 IL-17 RA 和 IL-17RC 的异源多聚体， 组成比例约为 2：1（1）。IL-17 RA 是一个约 130 kDa 的 I 型跨膜糖蛋白，且与 IL-17、肿瘤坏死因子或免疫球蛋白受体超家族成员没有相似性（2, 10, 13）。IL-17 RC 是 I 型跨膜蛋白，约为 90-95 kDa， 与 IL-17 RA 的氨基酸序列同源性仅为 30%（14, 15）。这两种受体对于 IL-17A 和 IL-17 A: F 的活性都是必须的。当配体与 IL-17RA 结合后， 这两种受体随后形成功能性结合 (1, 16)。

IL-17 主要参与中性粒细胞的召集和生存（3, 10, 17, 18）。其产生最早被认为是抗原刺激树突细胞的结果，导致IL-23 的分泌。在非T 细胞受体依赖的情况下，IL-23 会刺激T 细胞分泌IL-17（3）。分泌的IL-17 在骨髓中会刺激基质和成纤维细胞表达G-CSF 和膜式干细胞因子，并由此增加 PMN 的分化和形成。IL-17 通过阻断中性粒细胞的凋亡和促进 PMN 的体内循环，从而起到补充作用（17）。在组织中，主要通过对巨噬细胞和内皮细胞的作用，IL-17 可促进中性粒细胞的外渗。在巨噬细胞内，IL-17 可诱导TNF-α、IL-1β和IL-6 的产生(19)。TNF-α和IL-1β再作用于内皮细胞，并促进 G-CSF 的分泌，这一作用通过 IL-17 得到加强(20)。通过诱导内皮细胞 CXC 趋化因子和一氧化氮的释放，IL-17 起到了促进PMN 内流汇集，由此提高了血管的通透性（3, 9）。IL-17 的效应不仅仅局限于中性粒细胞，在关节部位，IL-17 可以上调破骨细胞中 RANKL 的表达量。这对破骨细胞的形成和随后的骨吸收起到了促进作用（18）。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗小鼠IL-17 单抗包被于微孔板上，样品和标准品中的IL-17 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IL-17 多抗，与结合在微孔板上的 IL-17 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小鼠血清；
- 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；
- 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）

已知浓度的两个样本，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，在不同板中分别检测 20 次，以确定板间精确度。

	板内精确度		板间精确度		
样本	1	2	1	2	3
平均值 (pg/mL)	40.6	432	41.2	51.9	480
标准差	3.5	26.2	3.7	4.6	40.9
CV%	8.6	6.1	9.0	8.9	8.5

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小鼠IL-17，测定其回收率。回收率范围在 82-110%， 平均回收率在 94%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠IL-17，测定其回收率。回收率范围在 79.5-92.0%，平均回收率在86.1%。

C. 灵敏度

小鼠IL-17 的最低可测值一般小于 1.8 pg/mL。

最低可测值是根据 20 个标准曲线零点吸光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R&D Systems®生产的大肠杆菌表达的高纯度重组小鼠IL-17蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠 IL-17，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	107	100 - 111
1:4	109	104 - 119
1:8	107	95 - 120
1:16	102	88 - 120

F. 样本预值

细胞培养上清液 - 从两个小鼠脾脏组织匀浆得到的原代细胞培养于100 mL 的 RPMI1640培养基中，细胞培养基还含有 10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL 青霉素、

100μg/mL 硫酸链霉素、10 μg/mL Con A，培养 2 天。取细胞培养上清液测定小鼠IL-17 含量，结果为 3424 pg/mL.

EL-4 细胞（小鼠胸腺瘤）以 2×10^5 细胞/mL 植培，培养 4 天；培养基为 100 mL 的DMEM 含有 10% 马血清、10 μg/mL PHA 和 10 ng/mL PMA。取细胞培养上清液测定得小鼠IL-17 含量，结果为 17 pg/mL。

血清样本 - 使用本试剂盒检测了4份小鼠血清样本中IL-17的水平。4份样本的检测值在33.5-46.0 pg/mL之间，平均值为38.4 pg/mL。

G. 特异性

此 ELISA 法可检测天然及重组小鼠IL-17 蛋白。将以下因子用稀释剂（1×）配置成 50ng/mL 的浓度来检测与小鼠IL-17 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IL-17 对照品中，来检测对小鼠 IL-17 的干扰。没有观察到明显的交叉反应或干扰。

重组小鼠蛋白

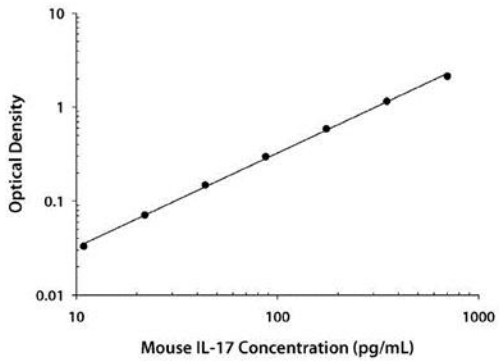
IL-17 B (aa 1-180)	IL-17 C
IL-17 B (aa 21-180)	IL-17 D
IL-17 BR	IL-17 E
IL-17 RC	IL-17 F
IL-17 RD	

小鼠IL-17 A/F 异源二聚体存在 66%的交叉反应。小鼠IL-17 R 浓度大于 10 ng/mL 时，会对检测造成影响。当浓度大于78 pg/mL时，重组小鼠IL-17 R1会干扰实验。

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)	O.D.	Average	Corrected
0	0.032 0.032	0.032	—
10.9	0.064 0.066	0.065	0.033
21.9	0.103 0.103	0.103	0.071
43.8	0.177 0.183	0.180	0.148
87.5	0.321 0.339	0.330	0.298
175	0.604 0.634	0.619	0.587
350	1.158 1.213	1.186	1.154
700	2.123 2.204	2.164	2.132

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-17 Microplate	包被抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse IL-17 Conjugate	酶标检测IL-17 抗体	1 瓶
Mouse IL-17 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 Covers	封板胶纸	3 张

* 本试剂盒包含足够的试剂以用于一块 96 孔微孔板的ELISA 实验。

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="8">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℃以下冰箱储存最多 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1000x 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×）5倍稀释后进行检测，即40μL血清+160μL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。

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 工作浓度的洗涤液。未用完的放回 4 ℃。

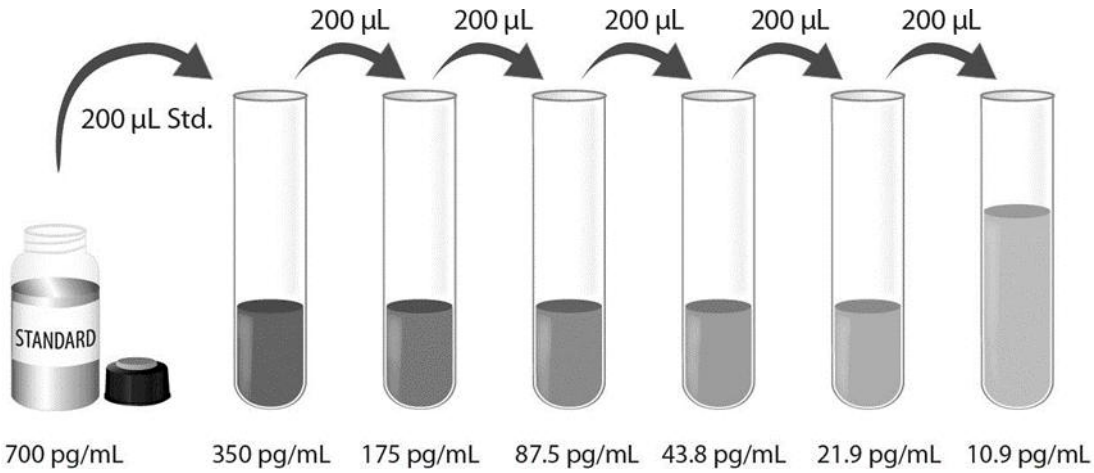
显色剂：按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合，避光；在使用前 15 分钟内准备，仅供当日使用；每孔需 100 μL。

稀释剂（1×）：可将 20mL 浓缩稀释剂用 80mL 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：参照标准品瓶身注明的方式重溶冻干标准品。得到浓度为 700pg/mL 标准品母液。轻轻震摇至少 5 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 200 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。没有稀释的标准品母液可用作标准曲线最高点（700pg/mL），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. 在每个微孔中加入 50 µL 稀释剂（1×）；
4. 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 50 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；
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。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 酶标检测抗体。用封板胶纸封住反应孔，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 显色底物，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，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；
10. 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570nm 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；
11. **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