PRODUCT INFORMATION & MANUAL

Mouse IL-17 Valukine™ ELISA

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

Version 202604.6

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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-17 RA and IL-17 RC, likely in a 2:1 ratio (1). IL-17 RA 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-17 RC is also a type I transmembrane protein, approximately 90-95 kDa in size, that shares less than 30% aa identity with IL-17 RA (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-17 RA (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. An 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 antibody specific for mouse IL-17 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 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 mouse serum, mouse 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 RD5T 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)	41.9	215	407	43.1	220	411
Standard Deviation	2.1	6.3	8.1	2.2	11.2	18.5
CV%	5.0	2.9	2.0	5.1	5.1	4.5

B. RECOVERY

The recovery of mouse IL-17 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)	101	92 - 118
Serum (n=6)	102	91 - 108
Heparin plasma (n=3)	101	91 - 123
EDTA plasma (n=3)	107	93 - 120

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-17 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 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 and/or spiked with various concentrations of mouse IL-17 and diluted with Calibrator Diluent RD5T to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	104	113	106	109
	Range (%)	101 - 106	107 - 118	98 - 111	107 - 110
1:4	Average % of Expected	98	106	108	111
	Range (%)	94 - 103	100 - 113	101 - 113	109 - 113
1:8	Average % of Expected	92	93	109	112
	Range (%)	83 - 97	85 - 104	94 - 119	108 - 114
1:16	Average % of Expected	87	86	107	109
	Range (%)	81 - 94	83 - 95	89 - 113	103 - 114

F. SAMPLE VALUES

Serum/Plasma - Forty mouse serum samples and six mouse plasma samples were evaluated for detectable levels of mouse IL-17 in this assay. All samples read below the lowest standard, 10.9 pg/mL.

Cell Culture Supernates - Mouse lung conditioned media (1 lung, 1-2 mm pieces in 10 mL DMEM containing 10% fetal bovine serum and 5 µg/mL lipopolysaccharide) was collected after 6 days. The culture supernate was assayed for mouse IL-17 and measured 1800 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:			**Recombinant human:**
C10	IL-9	KC	IL-17
Eotaxin	IL-10	Leptin	
G-CSF	IL-10 R	LIF	
GM-CSF	IL-12	M-CSF	
IFN-γ	IL-13	MIP-1α	
IL-1α	IL-17B (aa 1-180)	MIP-1β	
IL-1β	IL-17B (aa 21-180)	MIP-2	
IL-1ra	IL-17B R	OSM	
IL-2	IL-17D	SCF	
IL-3	IL-17E	TNF-α	
IL-4	IL-17F	Tpo	
IL-5	IL-17 R	VEGF	
IL-6	IL-18		
IL-7	JE/MCP-1		

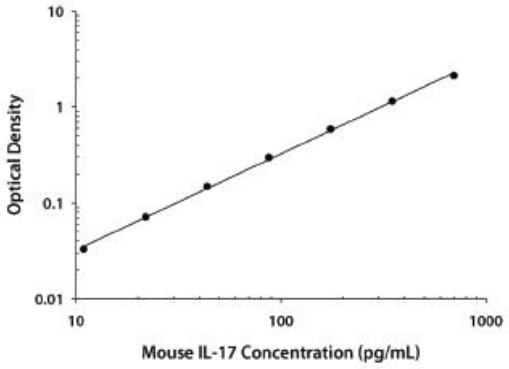
Some cross-reactivity was observed with recombinant mouse IL-17 A/F heterodimer.

Concentration Tested (pg/mL)	**Observed Value (pg/mL)**	**% Cross-Reactivity**
100	61	61

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 an antibody against mouse IL-17	1 plate
Mouse IL-17 Conjugate	Solution of an 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. Refer to the vial label for reconstitution volume.	1 vial
Assay Diluent RD1-38	A buffered protein solution with preservatives.	1 vial
Calibrator Diluent RD5T	A buffered protein solution with preservatives.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/ TMB Substrate Solution	1 vial
Stop Solution	Diluted hydrochloric acid solution	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>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>Assay Diluent RD1-38</td><td rowspan="2">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD5T</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

- 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 protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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 RD5T .

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 RD5T .

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5T.

Note: *Citrate plasma has not been validated for use in this assay.*

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

B. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

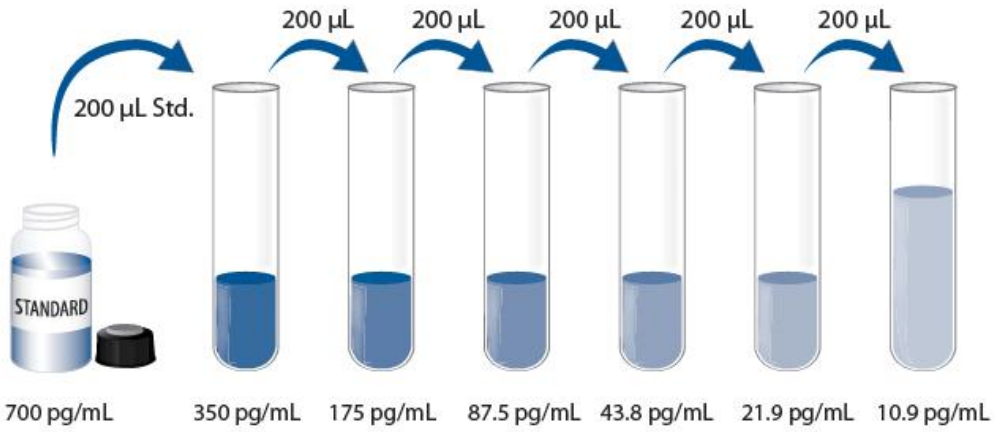
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Mouse IL-17 Standard - Refer to the vial label for reconstitution volume*. Reconstitute the Mouse IL-17 Standard with Calibrator Diluent RD5T. Do not substitute other diluents. 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 Calibrator Diluent RD5T 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 Calibrator Diluent RD5T 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

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, standards, 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 RD1-38 to each well.
4. Add 50 µL of Standard or 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 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 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 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 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.

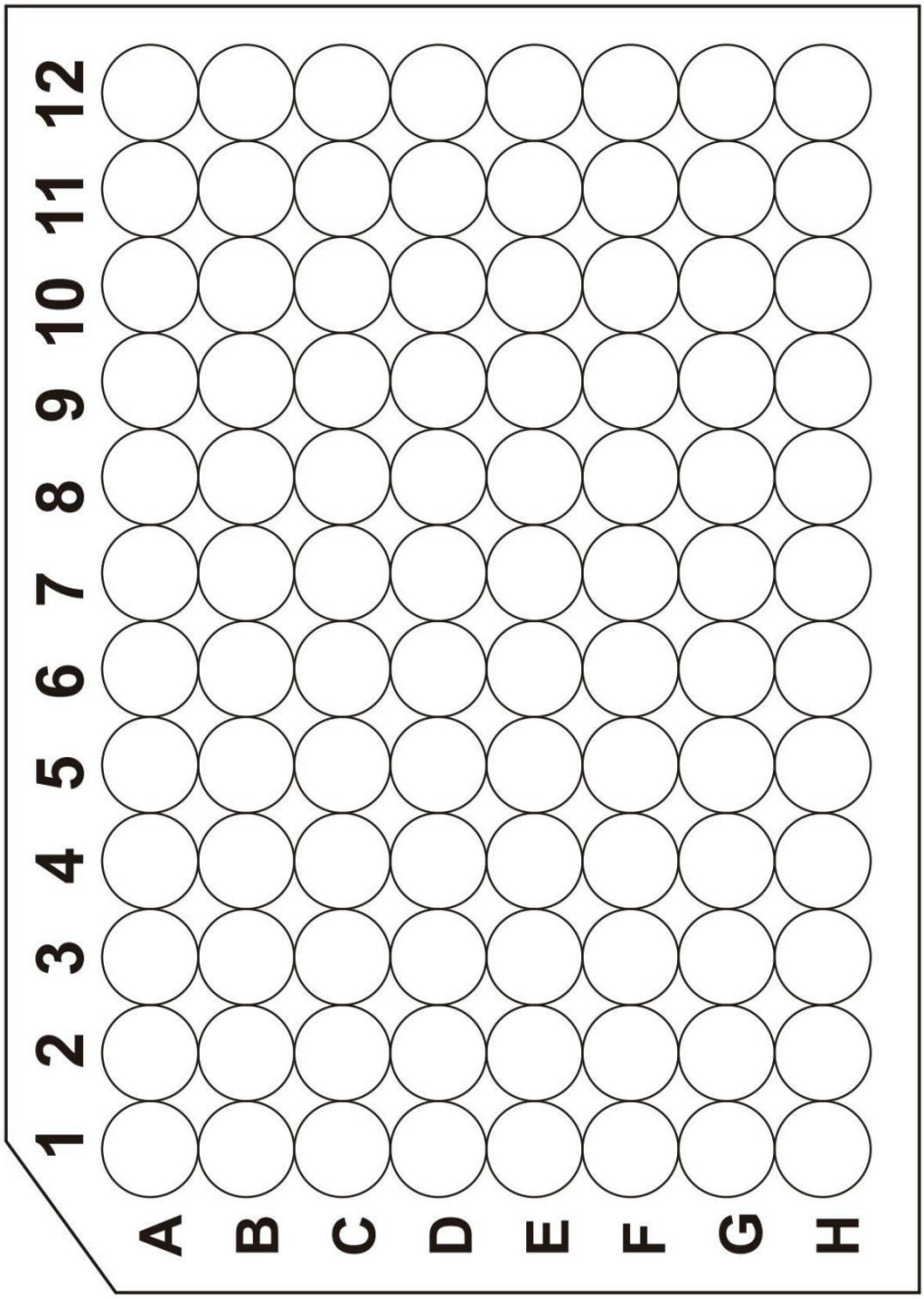
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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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

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版本号 202604.6

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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15 kDa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5 kDa。IL-17A可形成一个35-38 kDa的同源二聚体或与IL-17F形成一个45-48 kDa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-17 RC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不足30%（14, 15）。这两种受体对于IL-17A和IL-17A/F的活性都是必须的。当配体与IL-17 RA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抗体包被于微孔板上，样品和标准品中的小鼠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抗体，与结合在微孔板上的小鼠IL-17结合而形成免疫复合物，游离的成分被洗去；加入TMB底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；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和小鼠血清，小鼠血浆；
- 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RD5T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	3	1	2	3
平均值 (pg/mL)	41.9	215	407	43.1	220	411
标准差	2.1	6.3	8.1	2.2	11.2	18.5
CV%	5.0	2.9	2.0	5.1	5.1	4.5

B. 回收率

在不同样本中掺入检测范围内三种水平的小鼠IL-17，测定其回收率。

样品类型	平均回收率（%）	范围（%）
细胞培养上清（n=6）	101	92 - 118
血清（n=6）	102	91 - 108
肝素血浆（n=3）	101	91 - 123
EDTA血浆（n=3）	107	93 - 120

C. 灵敏度

小鼠IL-17的最低可测值一般小于5 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不同浓度的小鼠IL-17，然后用标准品稀释液RD5T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=4)	血清 (n=4)	EDTA血浆 (n=5)	肝素血浆 (n=5)
1:2	平均值/期待值（%）	104	113	106	109
	范围（%）	101 - 106	107 - 118	98 - 111	107 - 110
1:4	平均值/期待值（%）	98	106	108	111
	范围（%）	94 - 103	100 - 113	101 - 113	109 - 113
1:8	平均值/期待值（%）	92	93	109	112
	范围（%）	83 - 97	85 - 104	94 - 119	108 - 114
1:16	平均值/期待值（%）	87	86	107	109
	范围（%）	81 - 94	83 - 95	89 - 113	103 - 114

F. 样本预值

血清/血浆：在本检测中，评估了 40 份小鼠血清样本和 6 份小鼠血浆样本中小鼠 IL-17 的可检测水平。所有样本的读数均低于最低标准品（10.9 pg/mL）。

细胞培养上清：在培养6天后收集小鼠肺条件培养基（1个肺，切成1-2 mm的小块，置于10 mL含10%胎牛血清和5 μg/mL脂多糖的DMEM中）。检测该培养上清中的小鼠IL-17，测得结果为1800 pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠IL-17蛋白。将以下因子配制成50 ng/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。

Recombinant Mouse:			**Recombinant human:**
C10	IL-9	KC	IL-17
Eotaxin	IL-10	Leptin	
G-CSF	IL-10 R	LIF	
GM-CSF	IL-12	M-CSF	
IFN-γ	IL-13	MIP-1α	
IL-1α	IL-17B (aa 1-180)	MIP-1β	
IL-1β	IL-17B (aa 21-180)	MIP-2	
IL-1ra	IL-17B R	OSM	
IL-2	IL-17D	SCF	
IL-3	IL-17E	TNF-α	
IL-4	IL-17F	Tpo	
IL-5	IL-17 R	VEGF	
IL-6	IL-18		
IL-7	JE/MCP-1		

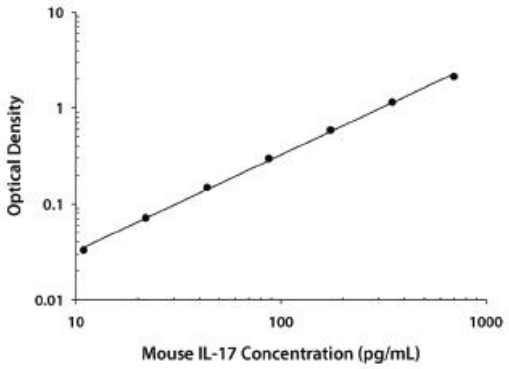
观察到与重组小鼠IL-17 A/F异源二聚体存在一定的交叉反应性。

检测浓度（pg/mL）	**观察值（pg/mL）**	**交叉反应性（%）**
100	61	61

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	0.032 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	包被抗小鼠 IL-17 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse IL-17 Conjugate	酶标 IL-17 检测抗体	1 瓶
Mouse IL-17 Standard	重组小鼠 IL-17 标准品（冻干），参考瓶身标签进行重溶	1 瓶
Assay Diluent RD1-38	一种含有防腐剂的缓冲蛋白溶液	1 瓶
Calibrator Diluent RD5T	一种含有防腐剂的缓冲蛋白溶液	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	1 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="4">2-8℃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>分装，-20℃冰箱储存最多 30 天*；避免反复冻融。</td></tr>
<tr><td>检测液 RD1-38</td><td rowspan="2">2-8℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释液 RD5T</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。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

以下样本采集和储存条件仅供一般性参考。样本稳定性尚未经过评估。

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。样本可能需要用标准品稀释液RD5T稀释。

血清样本：将血液样本在室温下静置凝集2小时，然后2000 × g离心20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。样本可能需要用标准品稀释液RD5T稀释。

血浆：使用EDTA或肝素作为抗凝剂采集血浆。采集后在30分钟内2000 × g离心20分钟。避免反复冻融。样本可能需用校准品稀释液RD5T进行稀释。

注意： *枸橼酸盐血浆尚未经验证可用于本检测。*

严重溶血或脂血样本不适用于本检测。

B. 检测前准备工作

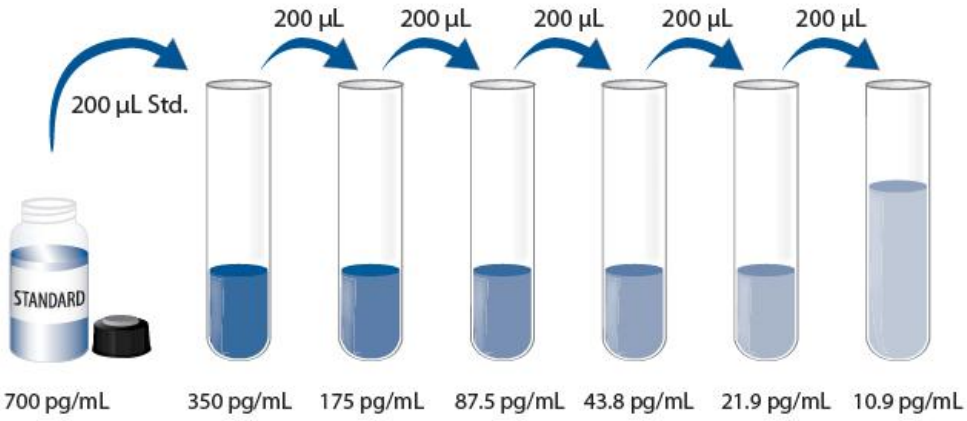
使用前请将所有试剂放置于室温。

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×）用去离子水或蒸馏水稀释配制成500 mL工作浓度的洗涤液（1×）。

小鼠IL-17标准品：参照标准品瓶身注明的方式重溶冻干标准品。使用标准品稀释液RD5T复溶小鼠IL-17标准品。请勿使用其他稀释液替代。溶解后得到浓度为700 pg/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标准品稀释液RD5T。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（700 pg/mL），标准品稀释液RD5T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、标准品和样品；
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检测液RD1-38；
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。轻轻敲击板框 1 分钟混合。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IL-17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

