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 202309.4

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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. 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, TMB substrate solution (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.
- 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

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
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 to 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 to 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 Calibrator 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 µg/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.

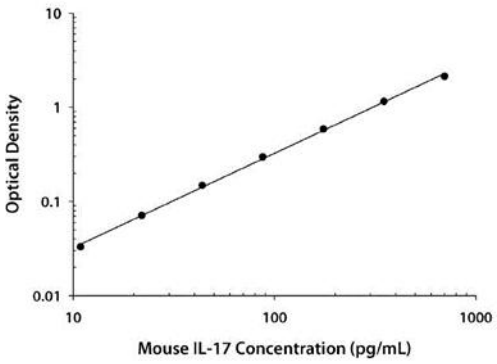
Recombinant Mouse:	
IL-17B (aa 1-180)	IL-17C
IL-17B (aa 21-180)	IL-17D
IL-17BR	IL-17E
IL-17 RC	IL-17F
IL-17 RD	

There is 66% cross-reactivity observed with recombinant mouse IL-17A/F Heterodimer. At concentration 10 ng/mL or greater, mouse IL-17 R 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. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (5×)	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	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="5">Opened/ Reconstituted Reagents</td><td>Diluted Wash Solution</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>Calibrator Diluent (5×)</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.
- 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

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×).

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×).

B. SAMPLE PREPARATION

Serum samples recommend a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

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

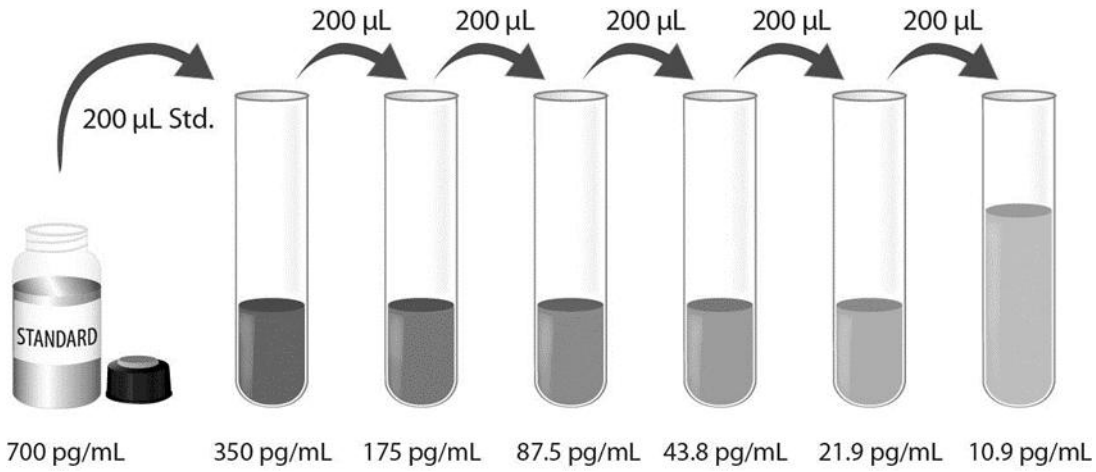
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse IL-17 Standard - Centrifuge briefly before opening. 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 15 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 (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 700 standard pg/mL serves as the high standard. The 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 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 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 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 Calibrator Diluent (1×) to each well.
4. Add 50 µL of Standard and sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.** A plate layout is provided for a record of standards and samples assayed.
5. Aspirate each well and wash, repeating the process 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 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.

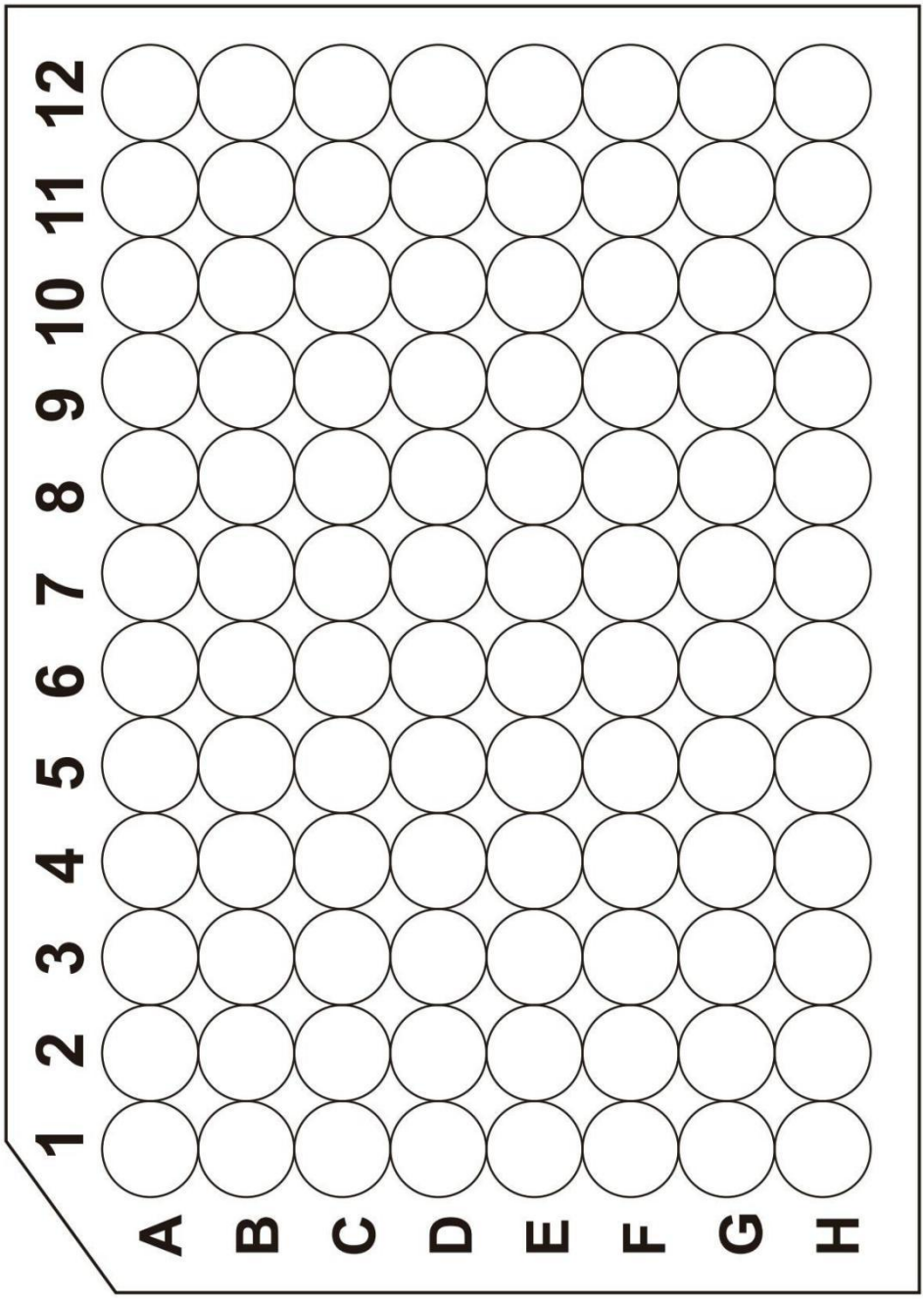
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Gaffen, S. (2009) Nat. Rev. Immunol. 9:556.
2. Iwakura, Y. et al. (2008) Immunol. Rev. 226:57.
3. Kolls, J.K. & A. Linden (2004) Immunity 21:467.
4. Yao, Z. et al. (1995) J. Immunol. 155:5483.
5. Kennedy, J. et al. (1996) J. Interf. Cytokine Res. 16:611.
6. Liang, S.C. et al. (2007) J. Immunol. 179:7791.
7. Chang, S. & C.Dong (2007) Cell Res. 17:435.
8. Rouvier, E. et al. (1993) J. Immunol. 150:5445.
9. Miljkovic, Dj. Et al. (2003) Cell. Mol. Life Sci. 60:518.
10. Witowski, J. et al. (2004) Cell. Mol. Life Sci. 61:567.
11. Cua, D.J. & C.M. Tato (2010) Nat. Rev. Immunol. 10:479.
12. Shin, H.C. et al. (1998) Cytokine 10:841.
13. Yao, Z. et al. (1995) Immunity 3:811.
14. Haudenschild, D. et al. (2002) J. Biol. Chem. 277:4309.
15. Toy, D. et al. (2006) J. Immunol. 177:36.
16. Hu, Y. et al. (2010) J. Immunol. 184:4307.
17. Schwarzenberger, P. et al. (2000) J. Immunol. 164:4783.
18. Yu, J.J. & S.L. Gaffen (2008) Front. Biosci. 13:170.
19. Jovanovic, D.V. et al. (1998) J. Immunol. 160:3513.
20. Numasaki, M. et al. (2004) Immunol. Lett. 95:97.

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 202309.4

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21kDa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 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-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仅为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；
- 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	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、100 µg/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。将以下因子用标准品稀释液（1×）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。

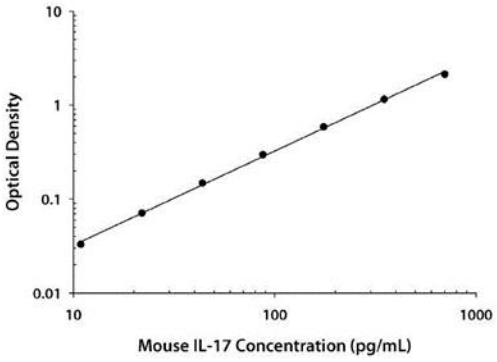
Recombinant Mouse:	
IL-17 B (aa 1-180)	IL-17 C
IL-17 B (aa 21-180)	IL-17 D
IL-17 BR	IL-17 E
IL-17 RC	IL-17 F
IL-17 RD	

小鼠IL-17A/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），参考瓶身标签进行重溶	1 瓶
Calibrator Diluent（5×）	浓缩的标准品稀释液（5×）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	1 瓶
Stop Solution	终止液	1 瓶
Plate Covers	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="6">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（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℃以下冰箱储存最多 30 天*；避免反复冻融。</td></tr>
<tr><td>标准品稀释液（5×）</td><td>2-8℃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℃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

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（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1000 × 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。样本可能需要用标准品稀释液（1×）稀释。

B. 样本准备工作

血清样本建议用标准品稀释液（1×）5倍稀释后进行检测，即40 µL血清+160 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

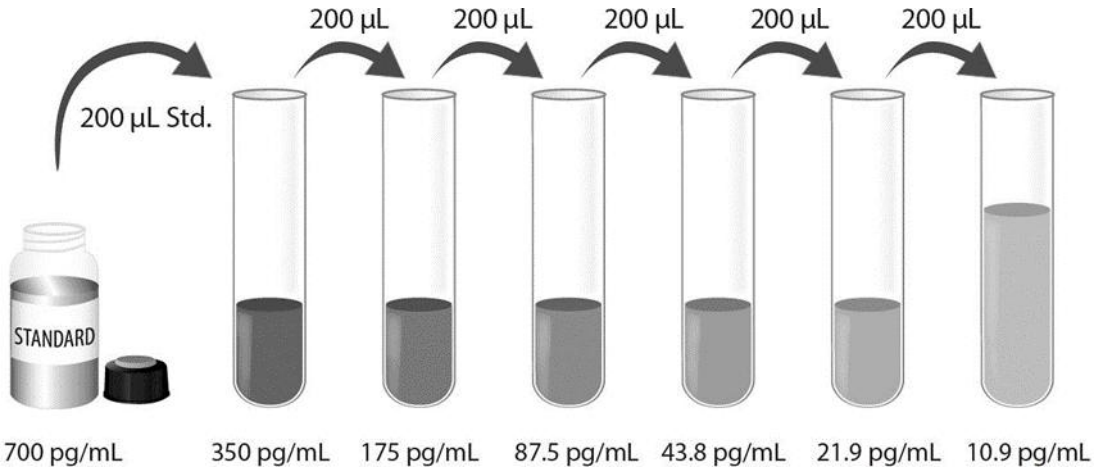
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液用去离子水或蒸馏水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液（1×）。

小鼠IL-17标准品：开盖前请瞬时离心。参照标准品瓶身注明的方式重溶冻干标准品。得到浓度为700 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。

每个稀释管中加入200 µL标准品稀释液（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。700 pg/mL管作标准曲线最高点，标准品稀释液（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标准品稀释液（1×）；
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。用封板膜 封住反应孔，**室温孵育2小时。**说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；（样本需要稀释，详情参见样本制备部分。）
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

1. Gaffen, S. (2009) Nat. Rev. Immunol. 9:556.
2. Iwakura, Y. et al. (2008) Immunol. Rev. 226:57.
3. Kolls, J.K. & A. Linden (2004) Immunity 21:467.
4. Yao, Z. et al. (1995) J. Immunol. 155:5483.
5. Kennedy, J. et al. (1996) J. Interf. Cytokine Res. 16:611.
6. Liang, S.C. et al. (2007) J. Immunol. 179:7791.
7. Chang, S. & C.Dong (2007) Cell Res. 17:435.
8. Rouvier, E. et al. (1993) J. Immunol. 150:5445.
9. Miljkovic, Dj. Et al. (2003) Cell. Mol. Life Sci. 60:518.
10. Witowski, J. et al. (2004) Cell. Mol. Life Sci. 61:567.
11. Cua, D.J. & C.M. Tato (2010) Nat. Rev. Immunol. 10:479..
12. Shin, H.C. et al. (1998) Cytokine 10:841.
13. Yao, Z. et al. (1995) Immunity 3:811.
14. Haudenschild, D. et al. (2002) J. Biol. Chem. 277:4309.
15. Toy, D. et al. (2006) J. Immunol. 177:36.
16. Hu, Y. et al. (2010) J. Immunol. 184:4307.
17. Schwarzenberger, P. et al. (2000) J. Immunol. 164:4783.
18. Yu, J.J. & S.L. Gaffen (2008) Front. Biosci. 13:170.
19. Jovanovic, D.V. et al. (1998) J. Immunol. 160:3513.
20. Numasaki, M. et al. (2004) Immunol. Lett. 95:97.

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