PRODUCT INFORMATION & MANUAL

Mouse IL-12 p70 Valukine™ ELISA

Catalog Number: VAL606

For the quantitative determination of natural and recombinant mouse Interleukin (IL)-12 p70 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 202609.7

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I. BACKGROUND

Interleukin 12 (IL-12, also NKSF) is a 70-75 kDa heterodimeric glycoprotein that belongs to the IL-12 family of heterodimeric cytokines (1-3). It consists of two disulfide-linked subunits which are 35 kDa (p35) and 40 kDa (p40) in size, and show no meaningful amino acid (aa) sequence identity (1, 4, 5). The mature p35 subunit is 196 aa in length and contains seven cysteines plus one potential N-linked glycosylation site (1-6). Mature mouse p35 shares 63% and 86% aa identity with human and rat p35, respectively (2, 7, 8). Mature mouse p40 is 313 aa in length, with 13 cysteines and five potential N-linked glycosylation sites. Polymorphisms have been reported in the p40 sequence, but these alleles are not recognized by the antibody pair used in this kit (9). Mature mouse p40 shares 72% and 93% aa identity with human and rat p40, respectively (1, 7, 10). While p35 resembles a hematopoietin ligand, p40 strongly resembles the N-terminus of a hematopoietin receptor, exhibiting a WSXWS motif, an immunoglobulin-like domain, and four conserved cysteines (1). This suggests that IL-12 may be a cytokine-receptor analog to the IL-6/soluble IL-6R complex (4, 6). Notably, while p40 may circulate as either a monomer or homodimer, p35 is never found by itself (3). p40 does, however, serve as the larger of two subunits that comprise IL-23 (3,11). Finally, while IL-12 is classically thought of as a secreted molecule, membrane-bound IL-12 has been reported on both human and mouse cells (12). Cells known to produce IL-12 include macrophages and dendritic cells (13), monocytes (14), Langerhans cells (15), neutrophils (16), keratinocytes (17), plasmacytoid dendritic cells (18), microglia (5), $CD8^+$ DC (mouse cells only) (19) and non-germinal center ($CD38^-$ $CD44^+$) B cells (human cells only) (3, 20).

The high affinity receptor for mouse IL-12 is composed of at least two type I transmembrane glycoproteins that resemble members of the cytokine receptor superfamily. The first subunit (Rα1) is 100 kDa in size and binds IL-12 with a Kd = 1 nM (21). This receptor serves as the principal binding site for the p40 subunit (4, 5). The second subunit (Rα2) is 130 kDa in size and shows no meaningful aa sequence identity to the Rα1 subunit (5, 21, 22). This receptor appears to be the

principal signal transduction component, and is suggested to serve as an attachment point for a disulfide-linked p35-p40 dimer (4, 5, 22). As noted above, mouse p40 will circulate either as a monomer, homodimer, or in a complex bound to either p35, forming IL-12, or to p19, forming IL-23 (3-5, 11). Both the homodimeric p40, and IL-23 can bind to the IL-12R, serving as nonsignalling antagonists (3, 23, 24). Alternatively, the p40 homodimer may also bind to Rα1, activating microglia and macrophages (4, 25).

Functionally, IL-12 has been shown to both enhance cytotoxic activity and induce interferon-gamma (IFN-γ) production in NK cells, T cells and dendritic epidermal T cells (3, 26-28). IL-12 has also been reported to induce IFN-γ production in macrophages (29). IL-12, in conjunction with the other IL-12 family members IL-23 and IL-27, is now believed to promote the development of a $CD4^+$ Th1 immune response (4, 5, 30). In response to infection, IL-27 is released initially, promoting a Th0 to Th0/1 transition state. IL-12 follows next, generating Th1 effector cells. With IL-18, IL-12 creates Th1 memory cells out of effector cells, and these cells are later activated by IL-23 (4).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse IL-12 p70 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IL-12 p70 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse IL-12 p70 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-12 p70 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 RD5Yand 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 separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	28	68	193	20	56	230
Standard Deviation	2.5	6.5	19.4	2.3	6.2	20.0
CV%	8.9	9.6	10.1	11.5	11.1	8.7

B. RECOVERY

The recovery of mouse IL-12 p70 spiked to three levels throughout the range of the assay was evaluated

Sample Type	**Average % Recovery**	**Range%**
Cell culture supernates (n=7)	101	95-106
Serum (n=7)	97	88-104

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-12 p70 is typically less than 2.5 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 recombinant mouse IL-12 p70 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-12 p70 and diluted with Calibrator Diluent RD5Yto produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=3)	**Serum (n=3)**
1:2	Average % of Expected	105	104
	Range (%)	101-108	104-105
1:4	Average % of Expected	105	103
	Range (%)	102-110	100-106
1:8	Average % of Expected	108	109
	Range (%)	106-110	106-113
1:16	Average % of Expected	105	107
	Range (%)	104-108	105-110

F. SAMPLE VALUES

Cell Culture Supernates - Two spleen organ tissues from a SJL mouse were homoginized and seeded at 1 x 10^6 cells/mL in RPMI supplemented with 10% fetal calf serum, 2 mM L-glutamine,100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 100 ng/mL mIFN-γ and 1 µg/mL LPS for 2-4 days. The cell culture supernate was assayed for mouse IL-12 p70 and measured 59 pg/mL.

Bone marrow mast cells collected from femurs of SJL mice were cultured (1 x 10^5 cells/mL) in RPMI supplemented with 10% fetal bovine serum and 25 ng/mL rmSCF. Recombinant mouse IFN-γ (100 ng/mL) was added on day 12 and LPS (1 µg/mL) was added on day 13. At day 15 the cell culture supernate was tested for mouse IL-12 p70

and measured 101 pg/mL.

Mouse serum - Forty mouse serum samples were evaluated for the presence of IL-12 p70 in this assay. Thirty-nine samples read below the lowest standard, 7.81 pg/mL. One sample read 21 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse IL-12 p70. The following factors were prepared at 50 ng/mL in Calibrator Diluent RD5Y and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse IL-12 p70 control were assayed for interference. No significant cross-reactivity or interference was observed.

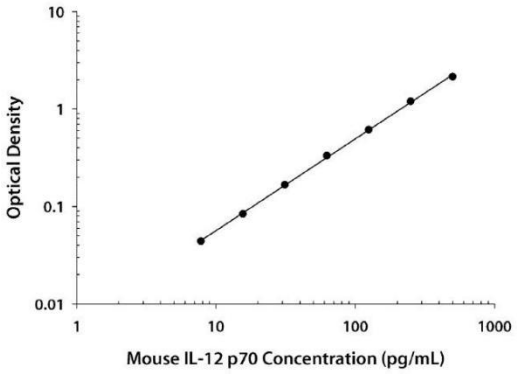
Recombinant mouse		Recombinant human
C10	IL-12/IL-23 p40 monomer	G-CSF
Eotaxin	IL-12/IL-23 p40 dimer	IL-6
G-CSF	IL-13	IL-6 R
GM-CSF	IL-23	IL-12 p35
IFN-γ	JE/MCP-1	IL-12/IL-23 p40 monomer
IL-1α	KC	IL-12 p70
IL-1β	Leptin	IL-23
IL-2	LIF	
IL-3	M-CSF	
IL-4	MIP-1α	
IL-5	MIP-1β	
IL-6	MIP-2	
IL-7	SCF	
IL-9	TNF-α	
IL-10	Tpo	
IL-10 R	VEGF	
IL-12 p35		

Recombinant rat IL-12 cross-reacts approximately 48% 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	0.012 0.013	0.012	—
7.81	0.053 0.058	0.056	0.044
15.6	0.096 0.097	0.096	0.084
31.3	0.177 0.181	0.179	0.167
62.5	0.343 0.347	0.345	0.333
125	0.616 0.636	0.626	0.614
250	1.193 1.229	1.211	1.199
500	2.157 2.172	2.164	2.152

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IL-12 p70 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse IL-12 p70	1 plate
Mouse IL-12 p70 Conjugate	Antibody against mouse IL-12 p70 conjugated to horseradish peroxidase with preservatives	1 vial
Mouse IL-12 p70 Standard	Recombinant mouse IL-12 p70 in a buffered protein base, lyophilized	1 vial
Assay Diluent RD1-14	A buffered protein base with preservatives. May contain a precipitate. *Mix well before and during use.*	1 vial
Calibrator Diluent RD5Y	Abuffered protein base with preservatives 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.	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="7">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="5">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1-14</td></tr>
<tr><td>Calibrator Diluent RD5Y</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Prepare fresh for each assay.
Standards may be stored for up to 1 month at -20°C *.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.

D. PRECAUTION

- The Stop Solution provided with this kit is an acid solution.
- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- TMB substrate may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- 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.

Serum - Use a serum separator tube (SST) and allow samples to clot for2 hours at room temperature before centrifugation for 20 minutes at 2000 × g. Remove serum and assay immediately or aliquot and store samples at ≤-20 °C. Avoid repeated freeze-thaw cycles..

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

B. REAGENT PREPARATION

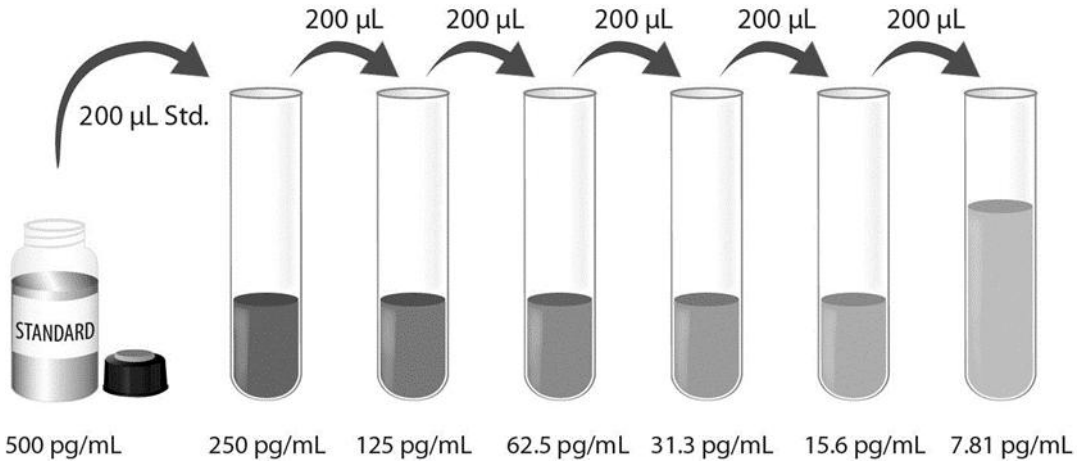
Note: *Bring all reagents to room temperature before use*.

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-12 p70 Standard - Refer to the vial label for reconstitution volume*.Reconstitute the Mouse IL-12 p70 Standard with Calibrator Diluent RD5Y. Do not substitute other diluents. This reconstitution produces a stock solution of 500 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.

Use polypropylene tubes . Pipette 200 µL of Calibrator Diluent RD5Y into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard 500 pg/mL serves as the high standard. The Calibrator Diluent RD5Y 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 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，samples 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 Assay Diluent RD1-14to each well. *Assay Diluent RD1-14 may contain undissolved material. Mix well before and during use.*
4. Add 50 µL of Standard 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 IL-12 p70 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. 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-12 p70 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.

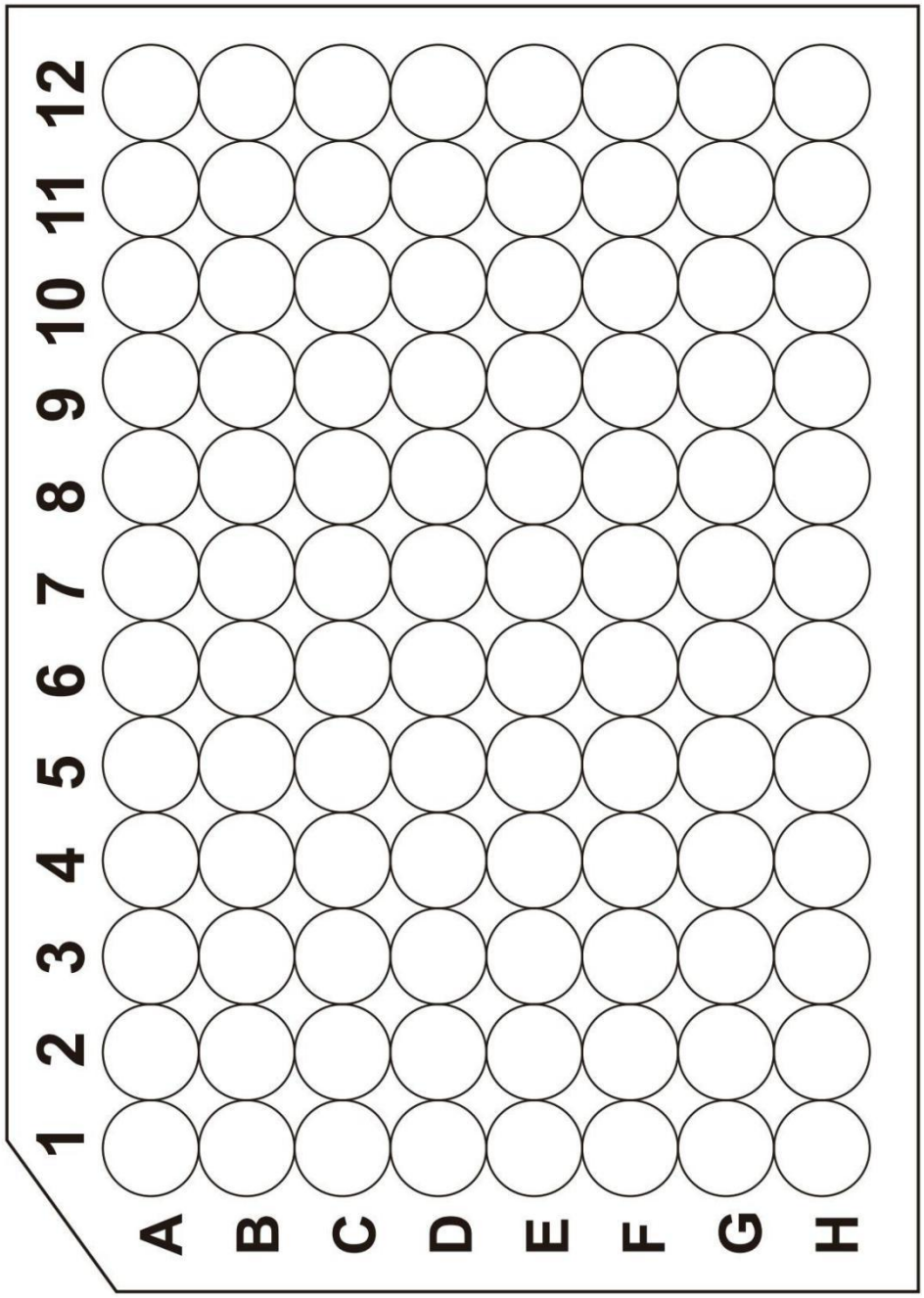
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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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-12 p70 Valukine™ ELISA 试剂盒

目录号：VAL606

适用于定量检测天然和重组小鼠白介素(IL)-12 p70 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

版本号 202609.7

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I. 背景

白细胞介素12（IL-12，也称NKSF），是70-75kDa的异源二聚体糖蛋白，属于IL-12异源二聚体细胞因子家族（1-3）。IL-12由35kDa(p35)和40kDa(p40)的两个亚基构成，这两个亚基由二硫键相连，相互间无氨基酸序列同源性（1, 4, 5）。成熟的p35亚基有196个氨基酸，包含七个半胱氨酸及一个潜在的N-连接糖基化位点（1-6）。成熟的小鼠p35与人和大鼠的氨基酸同源性分别为63%和86%。成熟的小鼠p40亚基长313个氨基酸，包括13个半胱氨酸和5个潜在的N-连接糖基化位点。有研究报道p40序列具有多态性，但R&D systems的这个IL-12 p70试剂盒不识别这些等位基因产物（9）。成熟的小鼠p40和人及大鼠的氨基酸同源性分别为72%和93%（1, 7, 10）。虽然p35类似于促红细胞生成素的配体，但p40更类似促红细胞生成素受体的N-端，有WSXWS结构域、一个免疫球蛋白样结构域和四个保守的半胱氨酸（1）。这表明IL-12可能是细胞因子受体模拟物，类似于IL-6可溶性IL-6R复合物（4, 6）。值得注意的是p40可以以单体或同源二聚体形式存在，而p35却从来没有发现单体形式（3）。p40是白介素23（IL-23）两个亚基中较大的亚基（3, 11）。虽然IL-12一直被认为是一种分泌型分子，人们在人和小鼠细胞中也发现了膜结合型IL-12（12）。产生IL-12的细胞包括巨噬细胞、树突状细胞（13）、单核细胞（14）、朗格汉斯细胞（15）、中性粒细胞(16)、角质形成细胞(17)、类浆树突状细胞（18）、小胶质细胞（5）、$CD8^+$ DC（仅小鼠细胞）（19）和非生发中心的（$CD38^-$ $CD44^+$） B细胞（仅人体细胞）（3, 20）。

小鼠IL-12的高亲和力受体是由至少2个I型跨膜糖蛋白组成，类似于细胞因子受体超家族成员。第一个亚基（Rα1）为100 kDa，以Kd=1nM与IL-12结合（21）。这个受体是p40亚基的主要结合位点（4, 5）；第二个亚基（Rα2）为130kDa，与Rα1亚基无氨基酸同源性（5, 21, 22）。这个受体是信号转导的重要组成部分，作为一个二硫键相连的p30-p40二聚体的附着点（4, 5, 22）。如上所述，小鼠p40以单体、二聚体形式存在，或于p35结合形成IL-12，或于p19结合形成IL-23(3-5, 11)。同源二聚体p40和IL-23都可以结合IL-12R，作为不传递信号的拮抗剂（3, 23, 24）。或者p40同源二聚体也可以与Rα1结合，激活小胶质细胞和巨噬细胞（4, 25）。

从功能上而言，IL-12已被证明能够增强细胞毒性、诱导NK细胞、T细胞和树突状表皮T细胞产生γ干扰素(3, 26, 27, 28)。有报道表明，IL-12能诱导巨噬细胞产生γ干扰素(29)。与IL-23和IL-27等其他IL-12的家族成员一起，IL-12能促进$CD4^+$ Th1型免疫反应的进展（4, 5, 30）。作为感染应答，IL-27最先分泌，促进TH0向THR0/1过渡，随后分泌IL-12，产生Th1效应细胞。与IL-18、IL-12一起产生把效应细胞转化Th1记忆细胞，接着这些细胞被IL-23激活（4）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠IL-12 p70抗体包被于微孔板上，样品和标准品中的小鼠IL-12 p70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-12 p70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，应将样本用稀释液RD5Y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，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)	28	68	193	20	56	230
标准差	2.5	6.5	19.4	2.3	6.2	20.0
CV%	8.9	9.6	10.1	11.5	11.1	8.7

B. 回收率

在样本中掺入检测范围内三个水平的小鼠IL-12 p70，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基 (n=7)	101	95-106
血清(n=7)	97	88-104

C. 灵敏度

小鼠IL-12 p70的最低可测剂量（MDD）一般小于2.5 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

此ELISA试剂盒经R&D Systems®生产的高纯度重组小鼠IL-12 p70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-12 p70，然后用标准品稀释液RD5Y将样本稀释到检测范围内，测定其线性。

		细胞培养上清（n=3）	血清（n=3）
1:2	平均值/期待值（%）	105	104
	范围（%）	101-108	104-105
1:4	平均值/期待值（%）	105	103
	范围（%）	102-110	100-106
1:8	平均值/期待值（%）	108	109
	范围（%）	106-110	106-113
1:16	平均值/期待值（%）	105	107
	范围（%）	104-108	105-110

F. 样本预值

细胞培养上清液- 小鼠脾细胞（1×10^6 细胞/mL）在含有 10% 胎牛血清、100 ng/mL 小鼠 IFN-γ 和 1 μg/mL LPS 的 RPMI 培养基中培养 2–4 天。取细胞培养上清检测小鼠 IL-12 p70，测得值为 59 pg/mL

来自SJL小鼠股骨骨髓的肥大细胞，以1×10^5细胞/mL的浓度，培植于含有10%胎牛血清的RPMI培养基中，培养4天；培养基还含有25 ng/mL重组小鼠SCF。第12天，添加100 ng/mL重组小鼠γ干扰素；第13天,添加1 μg/mL LPS。第15天取细胞上清测定IL-12 p70的含量，结果为101 pg/mL。

小鼠血清样本 - 使用本试剂盒检测了40份小鼠清样本中IL-12 p70的水平。39个样本的检测值均低于最低标准品，7.81 pg/mL，1个样本读数为21pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠IL-12 p70蛋白。将以下因子用标准品稀释液RD5Y配制成50 ng/mL的浓度来检测与小鼠IL-12 p70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-12 p70对照品中，来检测对小鼠IL-12 p70的干扰。没有观察到明显的交叉反应或干扰。

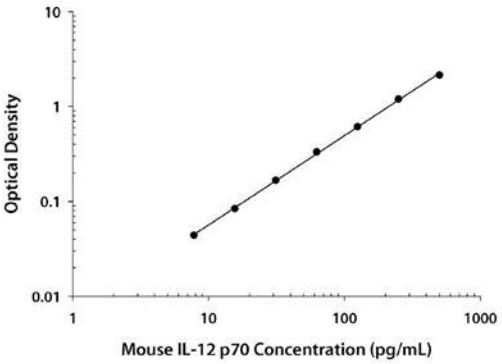
Recombinant mouse		Recombinant human
C10	IL-12/IL-23 p40 monomer	G-CSF
Eotaxin	IL-12/IL-23 p40 dimer	IL-6
G-CSF	IL-13	IL-6 R
GM-CSF	IL-23	IL-12 p35
IFN-γ	JE/MCP-1	IL-12/IL-23 p40 monomer
IL-1α	KC	IL-12 p70
IL-1β	Leptin	IL-23
IL-2	LIF	
IL-3	M-CSF	
IL-4	MIP-1α	
IL-5	MIP-1β	
IL-6	MIP-2	
IL-7	SCF	
IL-9	TNF-α	
IL-10	Tpo	
IL-10 R	VEGF	
IL-12 p35		

重组大鼠 IL-12 在该检测中约有48%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	0.012 0.013	0.012	—
7.81	0.053 0.058	0.056	0.044
15.6	0.096 0.097	0.096	0.084
31.3	0.177 0.181	0.179	0.167
62.5	0.343 0.347	0.345	0.333
125	0.616 0.636	0.626	0.614
250	1.193 1.229	1.211	1.199
500	2.157 2.172	2.164	2.152

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IL-12 p70 Microplate	包被抗小鼠IL-12 p70抗体的96孔聚苯乙烯板，8孔×12条	1块板
Mouse IL-12 p70 Conjugate	酶标检测小鼠IL-12 p70抗体	1瓶
Mouse IL-12 p70 Standard	重组小鼠IL-12 p70标准品（冻干），溶于含防腐剂的缓冲蛋白基质中。*参考瓶身标签进行重溶*	1瓶
Assay Diluent RD1-14	含防腐剂的缓冲蛋白基质。	1瓶
Calibrator Diluent RD5Y	含防腐剂的缓冲蛋白基质。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	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="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="5">2-8℃储存，最多30天*。</td></tr>
<tr><td>检测液RD1-14</td></tr>
<tr><td>标准品稀释液RD5Y</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><td>分装并于-20℃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；
- 用于稀释标准品的聚丙烯试管

D. 注意事项

- 试剂盒提供的终止液为酸性溶液。
- 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。
- TMB 底物溶液可能造成皮肤，眼睛和呼吸道刺激，避免吸入
- 穿戴防护手套、防护服、眼罩和面罩。操作后彻底清洗双手。

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。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集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。

注意：严重溶血或脂血样本可能不适用于本检测。

B. 试剂准备工作

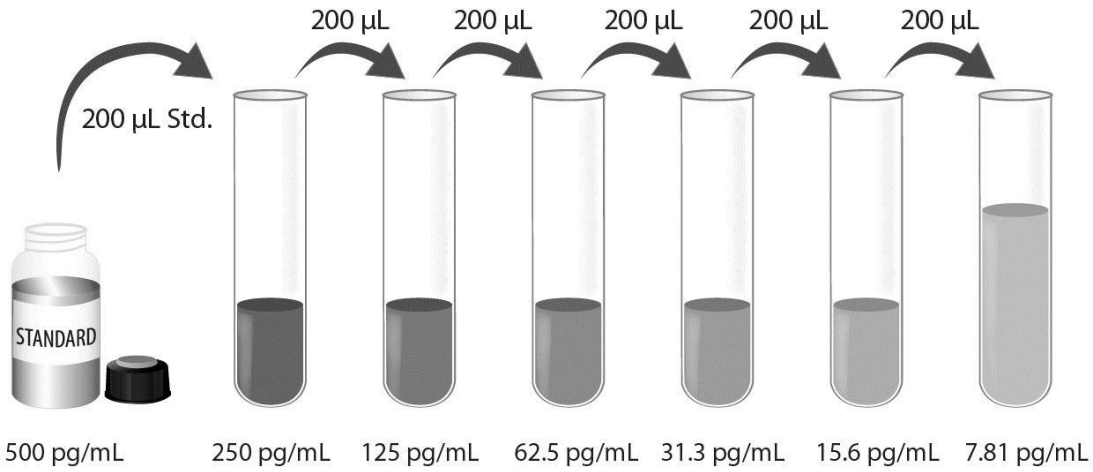
使用前请将所有试剂放置于室温。

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IL-12 p70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 RD5Y 复溶小鼠 IL-12 p70 标准品。不得使用其他稀释液替代。得到浓度为500 pg/mL标准品母液。轻轻震摇至少5分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯试管。每个稀释管中加入200 µL标准品稀释液RD5Y。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（500 pg/mL），标准品稀释液RD5Y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，样本和标准品；
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检测液RD1-14。*检测液 RD1-14 中可能含有未溶解物，使用前及使用期间需充分混匀；*
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分钟以混合均匀。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-12 p70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。

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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

