PRODUCT INFORMATION & MANUAL

Mouse IL-12 p70 Valukine™ ELISA

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.

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Novus kits are guaranteed for 3 months from date of receipt

Version 201910.2

TABLE OF CONTENTS

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 this set of R&D antibodies (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 ($CD38\text{-}CD44^+$) 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 (Rβ1) 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 (Rβ2) is 130 kDa in size and shows no meaningful aa sequence identity to the Rβ1 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 Rβ1, 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. A monoclonal 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 polyclonal antibody specific for mouse IL-12 p70 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-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 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)	23.6	76.5	17.0	48.6	222
Standard Deviation	2.3	4.6	3.0	4.2	13.6
CV%	9.7	6.0	17.7	8.6	6.1

B. RECOVERY

The recovery of mouse IL-12 p70 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 83-107% with an average of 93%.

The recovery of mouse IL-12 p70 spiked to four levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 70.5-80.3% with an average of 76.2%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-12 p70 is typically less than 2.24 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 *Sf21*-expressed 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 Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution	**Average % of Expected**	**Range (%)**
1:2	106	99 - 113
1:4	105	98 - 107
1:8	104	95 - 112
1:16	99	93 - 107

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 RPMI1640 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 5 days. The cell culture supernate was assayed for mouse IL-12p70 and measured 75.5 pg/mL.

EL-4 cells (Mouse thymoma) were seeded at 2x10^5 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-12p70 and measured 11 pg/mL.

Bone marrow mast cells collected from femurs of SJL mice were cultured (1 x 10^5 cells/mL) in RPMI1640 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-12p70 and measured 280 pg/mL.

Serum - Four serum samples were evaluated for the presence of IL-12 p70 in this assay. All samples measured below the lowest standard, 7.8 pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse IL-12 p70. 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-12 p70 control were assayed for interference. No significant cross-reactivity or interference was observed.

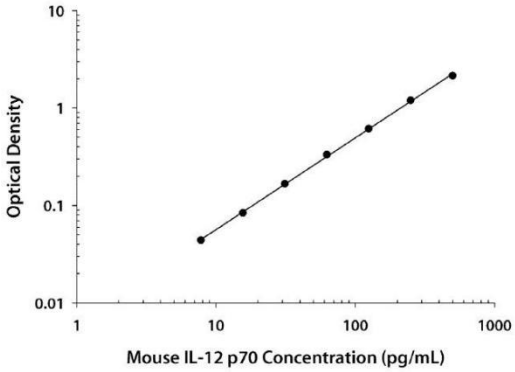
Recombinant mouse

IL-12p35	IL-12Rβ
IL-12p40 monomer	IL-12Rβ2
IL-12p40 dimer	

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 monoclonal antibody against mouse IL-12 p70	1 plate
Mouse IL-12 p70 Conjugate	Solution of a polyclonal antibody against mouse IL-12 p70 conjugated to horseradish peroxidase	1 vial
Mouse IL-12 p70 Standard	Recombinant mouse IL-12 p70 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 oil ouch 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 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 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.

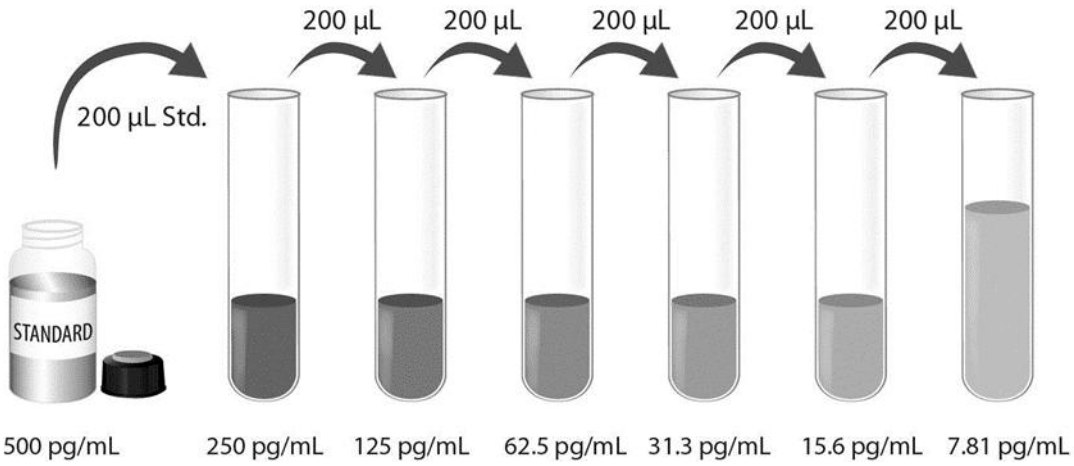
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

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-12 p70 Standard - Refer to the vial label for reconstitution volume*. 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.

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 500 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.*

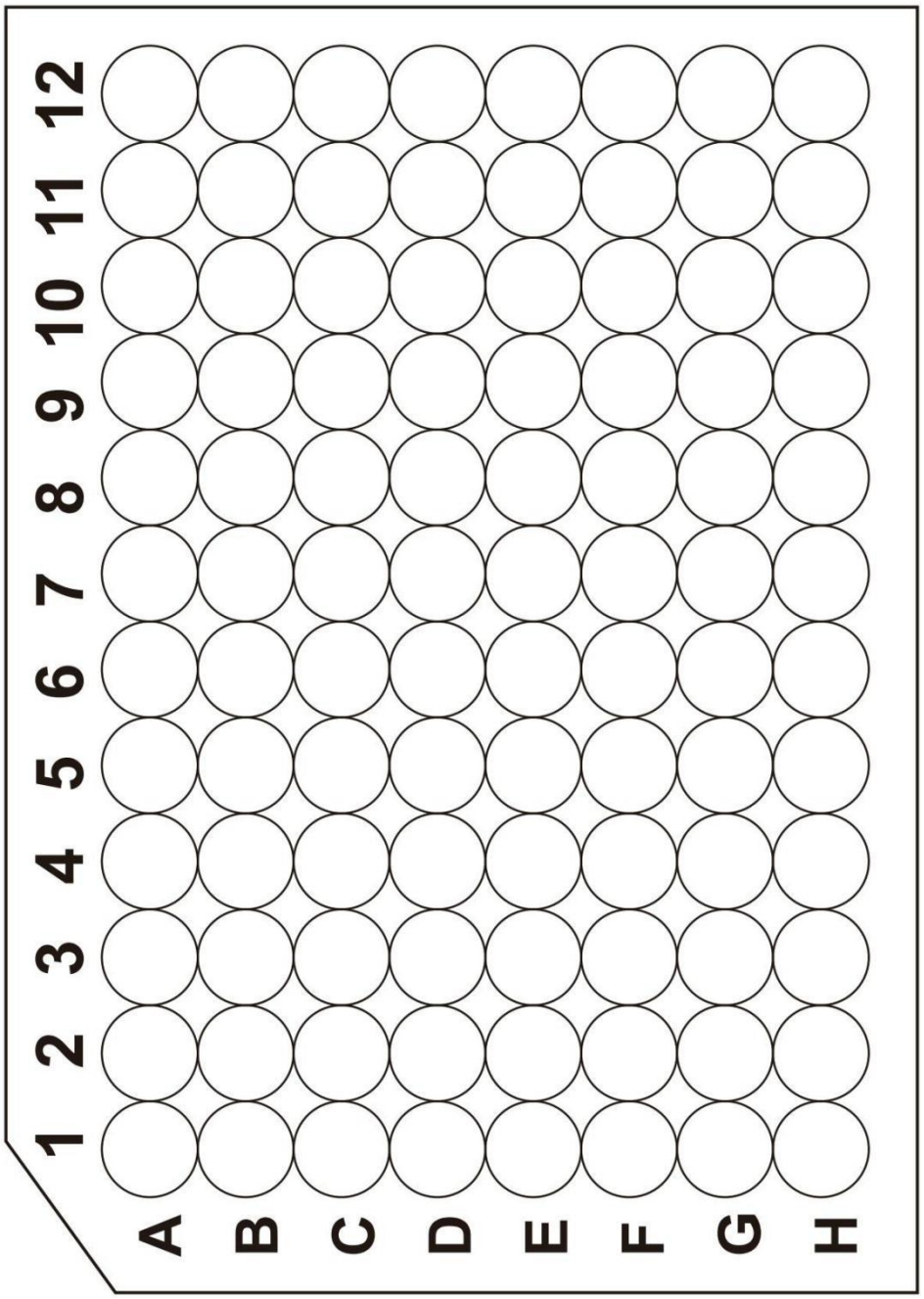
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Diluent 1× to each well.
4. Add 50 µL of Standard, sample, or control per 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-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 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-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.

VIII. REFERENCES

1. Sims, Schoenhaut, D.S. et al. (1992) J. Immunol. 148:3433.
2. Stern, A.S. et al. (1990) Proc. Natl. Acad. Sci. USA 87:6808.
3. Hamza, T. et al. (2010) Int. J. Mol. Sci. 11:789.
4. Brombacher, F. et al. (2003) Trends Immunol. 24:207.
5. Trinchieri, G. (2003) Nat. Rev. Immunol. 3:133.
6. Gearing, D.P. & D. Cosman (1991) Cell 66:9.
7. Gubler, U. et al. (1991) Proc. Natl. Acad. Sci. USA 88:4143.
8. Verma, N.D. et al. (2004) SwissProt Accession #:Q9R103.
9. Ymer, S.I. et al. (2002) Genes Immun. 3:151.
10. Khalife, J. et al. (1998) Eur. Cytokine Netw. 9:69.
11. Gee, K. et al. (2009) Inflamm. Allergy Drug Targets 8:40.
12. Fan, X. et al. (1996) Biochem. Biophys. Res. Commun. 225:1063.
13. Kato, T. et al. (1997) Cell. Immunol. 181:59.
14. Blotta, M.H. et al. (1997) J. Immunol. 158:5589.
15. Kang, K. et al. (1996) J. Immunol. 156:1402.
16. Romani, L. et al. (1997) J. Immunol. 158:5349.
17. Yawalkar, N. et al. (1996) J. Invest. Dermatol. 106:80.
18. Krug, A. et al. (2001) Eur. J. Immunol. 31:3026.
19. Shortman, K. & W. Heath (2010) Immunol. Rev. 234:18.
20. Schultze, J.L. et al. (1999) J. Exp. Med. 189:1.
21. Chua, A.O. et al. (1995) J. Immunol. 155:4286.
22. Presky, D.H. et al. (1996) Proc. Natl. Acad. Sci. USA 93:14002.
23. Heinzel, F.P. et al. (1997) J. Immunol. 158:4381.
24. Sieve, A.N. et al. (2010) Eur. J. Immunol. May 10. [Epub ahead of print].
25. Jana, M. et al. (2009) Glia 57:1553.
26. Novelli, F. & J.L. Casanova. (2004) Cytokine Growth factor Rev. 15:367.
27. Sugaya, M. et al. (1999) J. Invest. Dermatol. 113:350.
28. Tominaga, K. et al. (2000) Int. Immunol. 12:151.
29. Pudda, P. et al. (1997) J. Immunol. 159:3490.
30. Collison, L.W. & D. Vignali (2008) Immunol. Rev. 226:248.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠IL-12p70 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

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Novus 试剂盒确保在你收货日期 3 个月内有效

目录

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35 kDa(p35)和40 kDa(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（Rβ1）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（Rβ2）为130 kDa， 与Rβ1 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Rβ1 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 过度， 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 单抗包被于微孔板上，样品和标准品中的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 多抗，与结合在微孔板上的 IL-12 p70 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)	23.6	76.5	17.0	48.6	222
标准差	2.3	4.6	3.0	4.2	13.6
CV%	9.7	6.0	17.7	8.6	6.1

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-12 p70，测定其回收率。回收率范围在83-107%，平均回收率在 93%。

在小鼠清样本中掺入检测范围内不同水平的小鼠IL-12 p70，测定其回收率。回收率范围70.5-80.3%，平均回收率在76.2%。

C. 灵敏度

小鼠IL-12 p70 的最低可测剂量（MDD）一般小于 2.24 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®生产的大肠杆菌表达的高纯度重组小鼠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	106	99 - 113
1:4	105	98 - 107
1:8	104	95 - 112
1:16	99	93 - 107

F. 样本预值

细胞培养上清液- SJL 小鼠的两个脾脏匀浆后，以 1×10^6 细胞/mL 的浓度；培植于含有 10%胎牛血清的RPMI1640 培养基中；培养五天；培养基同时还含有 2 mM L-谷氨酰胺、100U/mL 青霉素、100 µg/mL 链霉素、100 ng/mL 小鼠γ干扰素和I µg/mL LPS。取细胞上清液测量IL-12p70 的含量，结果为 75.5 pg/mL。

EL-4 细胞（小鼠胸腺瘤），以 2×10^5 细胞/mL 的浓度，在 100 mL 的含有 10%马血清的

DMEM 培养基中培养 4 天，培养基同时还含有 10 µg/mL PHA、10 ng/mL PMA。取细胞上清液测量IL-12p70 的含量，结果为 11 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 RPMI1640 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-12p70 的含量，结果为 280 pg/mL。

血清样本 - 使用本试剂盒检测了4份小鼠清样本中 IL-12 p70 的水平。4份样本的检测值均低于最低标准品，7.8pg/mL。

G. 特异性

此 ELISA 法可检测天然及重组小鼠IL-12 p70 蛋白。将以下因子用稀释剂（1×）配置成 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。

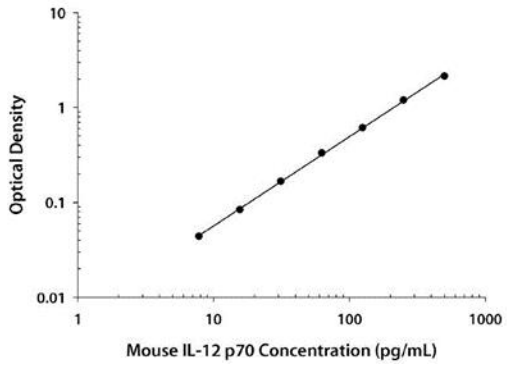
重组小鼠蛋白

IL-12p35	IL-12Rβ
IL-12p40 monomer	IL-12Rβ2
IL-12p40 dimer	

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	0.012 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IL-12 p70 抗体	1 瓶
Mouse IL-12 p70 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="7">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1000 x 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×）2倍稀释后进行检测，即100 µL血清+100 µL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℃。

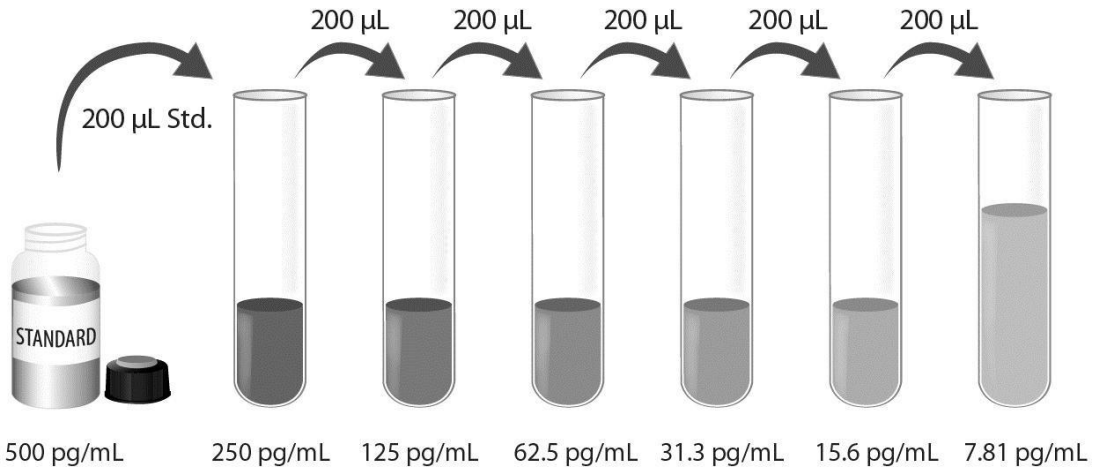
显色剂：按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合，避光；在使用前15 分钟内准备，仅供当日使用；每孔需 100 µL。

稀释剂（1×）：可将 20 mL 浓缩稀释剂用 80 mL 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 500 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 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（500pg/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

1. Sims, Schoenhaut, D.S. et al. (1992) J. Immunol. 148:3433.
2. Stern, A.S. et al. (1990) Proc. Natl. Acad. Sci. USA 87:6808.
3. Hamza, T. et al. (2010) Int. J. Mol. Sci. 11:789.
4. Brombacher, F. et al. (2003) Trends Immunol. 24:207.
5. Trinchieri, G. (2003) Nat. Rev. Immunol. 3:133.
6. Gearing, D.P. & D. Cosman (1991) Cell 66:9.
7. Gubler, U. et al. (1991) Proc. Natl. Acad. Sci. USA 88:4143.
8. Verma, N.D. et al. (2004) SwissProt Accession #:Q9R103.
9. Ymer, S.I. et al. (2002) Genes Immun. 3:151.
10. Khalife, J. et al. (1998) Eur. Cytokine Netw. 9:69.
11. Gee, K. et al. (2009) Inflamm. Allergy Drug Targets 8:40.
12. Fan, X. et al. (1996) Biochem. Biophys. Res. Commun. 225:1063.
13. Kato, T. et al. (1997) Cell. Immunol. 181:59.
14. Blotta, M.H. et al. (1997) J. Immunol. 158:5589.
15. Kang, K. et al. (1996) J. Immunol. 156:1402.
16. Romani, L. et al. (1997) J. Immunol. 158:5349.
17. Yawalkar, N. et al. (1996) J. Invest. Dermatol. 106:80.
18. Krug, A. et al. (2001) Eur. J. Immunol. 31:3026.
19. Shortman, K. & W. Heath (2010) Immunol. Rev. 234:18.
20. Schultze, J.L. et al. (1999) J. Exp. Med. 189:1.
21. Chua, A.O. et al. (1995) J. Immunol. 155:4286.
22. Presky, D.H. et al. (1996) Proc. Natl. Acad. Sci. USA 93:14002.
23. Heinzel, F.P. et al. (1997) J. Immunol. 158:4381.
24. Sieve, A.N. et al. (2010) Eur. J. Immunol. May 10. [Epub ahead of print].
25. Jana, M. et al. (2009) Glia 57:1553.
26. Novelli, F. & J.L. Casanova. (2004) Cytokine Growth factor Rev. 15:367.
27. Sugaya, M. et al. (1999) J. Invest. Dermatol. 113:350.
28. Tominaga, K. et al. (2000) Int. Immunol. 12:151.
29. Pudda, P. et al. (1997) J. Immunol. 159:3490.
30. Collison, L.W. & D. Vignali (2008) Immunol. Rev. 226:248.

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