PRODUCT INFORMATION & MANUAL

Mouse IFN-γ Valukine™ ELISA

Catalog Number: VAL607

For the quantitative determination of natural and recombinant mouse IFN-γ 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 202412.4

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I. BACKGROUND

Interferon-gamma (IFN-γ, also known as type II interferon) is an important immunoregulatory cytokine that was originally identified through its anti-viral activity (1, 2). It plays key roles in host defense by exerting antiviral, antiproliferative, and immunoregulatory activities (3, 4). On many cell types, IFN-γ induces the production of cytokines and upregulates the expression of various membrane proteins including class I and II MHC antigens, Fc receptors, leukocyte adhesion molecules, and B7 family antigens. IFN-γ is a potent activator of macrophage effector functions. It directs the synthesis, class switching, and secretion of immunoglobulins by B cells. IFN-γ also influences T-helper cell phenotype development by inhibiting Th2 differentiation and stimulating Th1 development (3, 4). IFN-γ plays a central role in the progression of inflammatory diseases such as autoimmunity and atherosclerosis (5, 6).

Biologically active IFN-γ consists of a noncovalently linked homodimer of 20-25 kDa variably glycosylated subunits (7). Mature mouse IFN-γ shares 86% amino acid (aa) sequence identity with rat IFN-γ and 38-44% aa identity with bovine, canine, cotton rat, equine, feline, human, porcine, and rhesus IFN-γ. IFN-γ dimers bind to transmembrane IFN-γ RI (alpha subunits) which then interact with transmembrane IFN-γ RII (beta subunits) to form the functional receptor complex of two α and two β subunits (8, 9). Inclusion of IFN-γ RII in the receptor complex increases the ligand binding affinity as well as the efficiency of signal transduction (9, 10). Whereas the α-chain is expressed constitutively on many cell types, the cellular regulation of the β-chain correlates with an IFN-γ responsive state and is tightly regulated (8).

IFN-γ is produced by a number of cell types under inflammatory conditions, including dendritic epidermal/γδ T cells (11), keratinocytes (12), peripheral blood γδ T cells (13), mast cells (14), neurons (15), $CD8^+$ T cells (16), macrophages (17), B cells (18), neutrophils (19), NK cells (20), $CD4^+$ T cells (21), and testicular spermatids (22).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse IFN-γ has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse IFN-γ present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse IFN-γ 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 IFN-γ 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 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)	22.9	345	25.3	128	344
Standard Deviation	1.0	9.9	3.8	11.2	39.7
CV%	4.4	2.9	15.2	8.8	11.5

B. RECOVERY

The recovery of mouse IFN-γ spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 89.0 to 110.0% with an average of 96.0%.

The recovery of mouse IFN-γ spiked to different levels throughout the range of the assay in mouse serum was evaluated. The recovery ranged from 74.0 to 85.3% with an average of 76.2%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IFN-γ is typically less than 2.1 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 IFN-γ 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 IFN-γ and diluted with Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	105	103 - 117
1:4	109	107 - 112
1:8	105	100 - 110
1:16	101	93 - 107

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 g/mL streptomycin sulfate, and 10 µg/mL Con A for 2 days. The cell culture supernate was assayed for mouse IFN-γ and measured 3248 pg/mL.

Mouse thymoma cells (EL-4, 2×10^5 cells/mL) were cultured in DMEM plus 10% Horse Serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA for 4 days. The cell culture supernate was assayed for mouse IFN-γ and measured 51 pg/mL.

CTLL-2 cells (5×10^4 cells/mL) were cultured for 3 days in RPMI plus 10% FBS, 2 mM L- glutamine, 10 ng/mL rmIL-2, 50 mM 2-mercaptoethanol and stimulated with 2.5 ng/mL LPS, and 100 ng/mL rmGM-CSF. Unstimulated is 95 pg/mL and stimulated is 306 pg/mL.

Mouse serum - Four mouse serum samples were evaluated for the presence of mouse IFN-γ in this assay. All samples measured ranged from 28.6 to 48.3 pg/mL with an average of 39.0 pg/mL.

G. SPECIFICITY

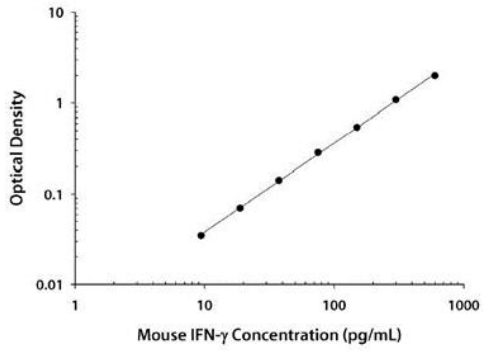
This assay recognizes both natural and recombinant mouse IFN-γ. The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse IFN-γ control were assayed for interference. No significant cross-reactivity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

Recombinant mouse:	
IL-10	IFN-γ R1
IFN-kappa	IFN-γ R2

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	0.042 0.043	0.042	—
9.4	0.077 0.077	0.077	0.035
18.8	0.113 0.112	0.112	0.070
37.5	0.183 0.186	0.184	0.142
75	0.327 0.329	0.328	0.286
150	0.580 0.594	0.587	0.545
300	1.130 1.124	1.127	1.085
600	2.020 2.042	2.031	1.989

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IFN-γ Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse IFN-γ	1 plate
Mouse IFN-γ Conjugate	Antibody against mouse IFN-γ conjugated to horseradish peroxidase	1 vial
Mouse IFN-γ Standard	Recombinant mouse IFN-γ in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Diluent (5×)/RD5P	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/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="6">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>Prepare fresh for each assay.
Standards may be stored for up to 1 month at -20°C *.</td></tr>
<tr><td>Diluent (5×)/RD5P</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Diluent (1×). Prepare fresh for each assay.</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. Samples may require dilution with 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 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 Diluent (1×).

B. SAMPLE PREPARATION

Mouse serum samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

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

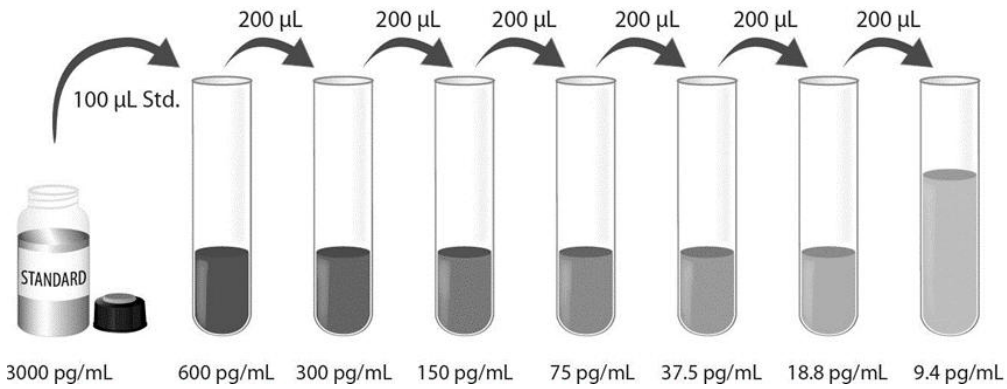
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse IFN-γ Standard - Centrifuge briefly before opening. Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 3000 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 400 µL of Diluent (1×) into the 600 pg/mL tube. Pipette 200 µL of Diluent (1×) into each remaining tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 600 pg/mL standard 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.
- 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 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 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 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 IFN-γ 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 IFN-γ concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

VIII. REFERENCES

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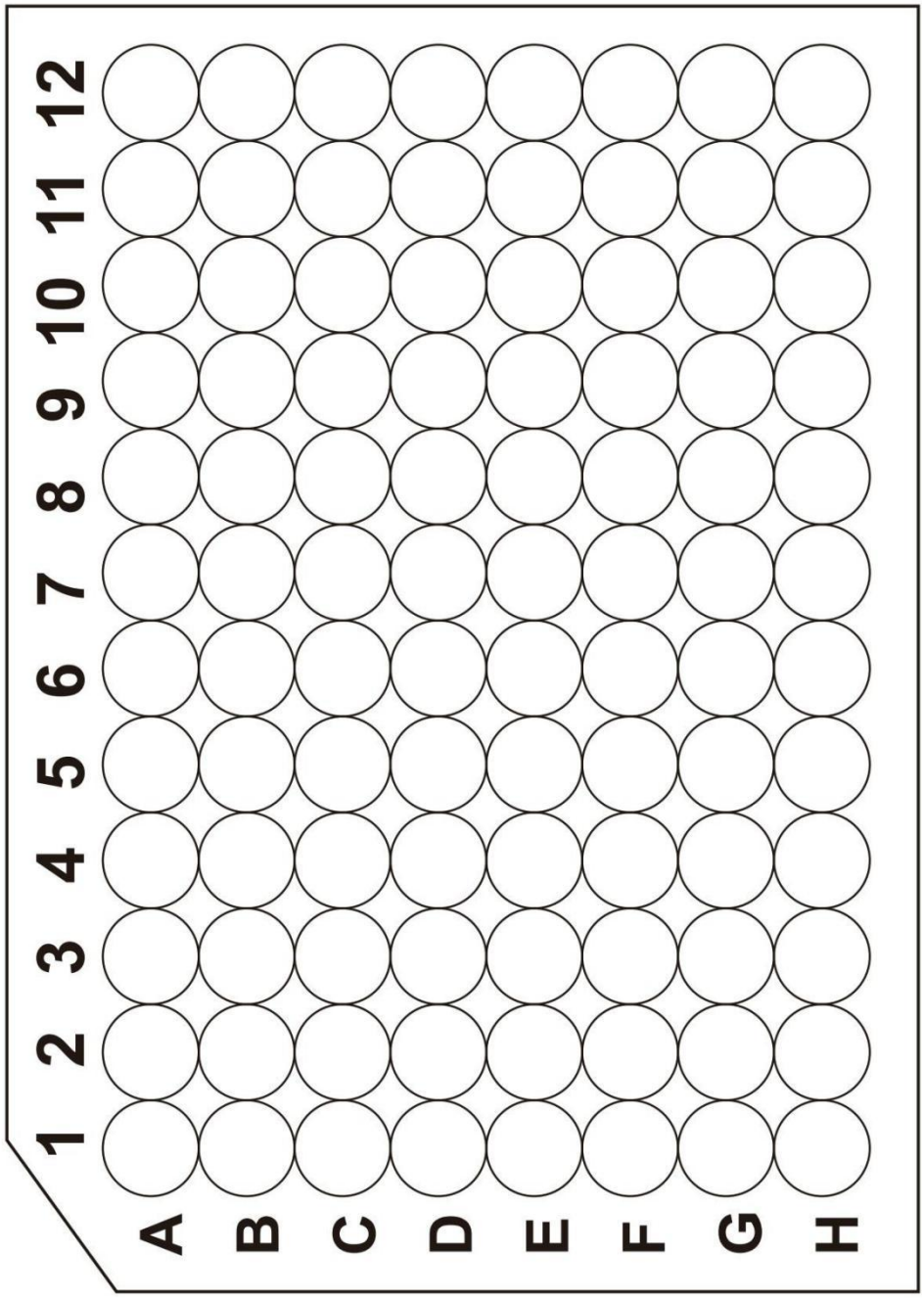
21. Briscoe, D.M. et al. (1997) J. Immunol. 159:3247.
22. Dejuco, N. et al. (1995) Endocrinology 136:4925.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 IFN-γ Valukine™ ELISA 试剂盒

目录号：VAL607

适用于定量检测天然和重组小鼠 IFN-γ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 202412.4

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I. 背景

γ干扰素（IFN-γ，又称为II型干扰素）是一种重要的免疫调节细胞因子，因其具有抗病毒活性而被发现（1, 2）。IFN-γ通过其抗病毒、抗增殖和免疫调节功能在宿主防御过程中起关键作用（3, 4）。在许多类型的细胞中，IFN-γ诱导细胞因子在生产和上调多种膜蛋白的表达，包括I型和II型主要组织相容性复合体（MHC）抗原、Fc受体、白细胞黏附分子和B7家族抗原。IFN-γ是强大的巨噬细胞效应激活剂；它指导B细胞免疫球蛋白的合成、类型转变及分泌。IFN-γ通过抑制Th2细胞的分化和刺激Th1细胞的发育而影响T辅助细胞表型的发育（3, 4）。IFN-γ在自身免疫疾病和动脉粥样硬化等炎性疾病的恶化过程中起重要作用（5, 6）。

具有生物活性的IFN-γ是由两个非共价相连的具有不同程度糖基化的20-25 kDa亚基组成的同源二聚体（7）。成熟的小鼠IFN-γ与大鼠IFN-γ在氨基酸序列上享有86%的同源性，与牛、犬、棉鼠、马、猫、人、猪及恒河猴的IFN-γ有38-44%的同源性。IFN-γ二聚体先于跨膜IFN-γ RI（α亚基）集合，再与跨膜IFN-γ RII(β亚基) 结合，从而形成含有两个 α亚基和β亚基的活性受体复合体（8, 9）。受体复合体中的IFN-γ RII可增加配体的亲和力以及信号转导的效率（9, 10）。尽管α链在多种细胞类型中广泛表达， 受体β链却与IFN-γ的应答状态相关，其表达受到严格调控（8）。

在炎症条件下，IFN-γ可由多种类型的细胞产生，包括树突状表皮/γδ T细胞（11）、角质形成细胞（12）、外周γδ T细胞（13）、肥大细胞（14）、神经元（15）、$CD8^+$ T细胞（16）、巨噬细胞（17）、B 细胞（18）、中性粒细胞（19）、自然杀伤细胞（20）、$CD4^+$ T细胞（21）和睾丸精子细胞（22）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠IFN-γ抗体包被于微孔板上，样品和标准品中的小鼠IFN-γ会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶偶联的抗小鼠IFN-γ抗体孵育；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；
- 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（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)	22.9	345	25.3	128	344
标准差	1.0	9.9	3.8	11.2	39.7
CV%	4.4	2.9	15.2	8.8	11.5

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IFN-γ，测定其回收率。回收率范围在89.0-110.0%，平均回收率在96.0%。

在小鼠血清样本中掺入检测范围内不同水平的小鼠IFN-γ，测定其回收率。回收率范围在74.0-85.3%，平均回收率在76.2%。

C. 灵敏度

小鼠IFN-γ的最低可测值一般小于2.1 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试剂盒以R&D Systems生产的大肠杆菌表达的高纯度重组小鼠IFN-γ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 IFN-γ，然后用稀释液（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	105	103 - 117
1:4	109	107 - 112
1:8	105	100 - 110
1:16	101	93- 107

F. 样本预值

细胞培养上清液：

将小鼠的两个脾脏组织匀浆后，培养在100 mL含10%胎牛血清、2 mM L-谷氨酸钠、100 U/mL青霉素, 100 g/mL硫酸链霉素、10 μg/mL ConA的RPMI1640培养基中，培养2天。取细胞培养上清液检测小鼠IFN-γ含量， 检测值为3248 pg/mL。

小鼠胸腺瘤细胞（EL-4，2×10^5 cells/mL）培养在100 mL含10%马血清的DMEM培养基中，用10μg/mL PHA和10 ng/mL PMA刺激细胞，培养4天；检测细胞培养上清液中小鼠IFN-γ含量，检测值为51 pg/mL。

CTLL-2细胞（5×10^4 cells/mL）培养养在含10%胎牛血清、2 mM L-谷氨酸钠、50 mM巯基乙醇、10 ng/mL重组小鼠IL-2 RPMI 1640培养基中，不刺激细胞或用100 ng/mL重组小鼠GM-CSF、2.5 ng/mL LPS刺激细胞，培养3天；取细胞上清检测小鼠IFN-γ含量，不刺激的检测值为95 pg/mL; 刺激的检测值为306 pg/mL。

小鼠血清样本-用本试剂盒检测了4份小鼠血清样本中IFN-γ的水平。所有样本的检测值在28.6-48.3 pg/mL之间，平均值为39.0 pg/mL。

G. 特异性

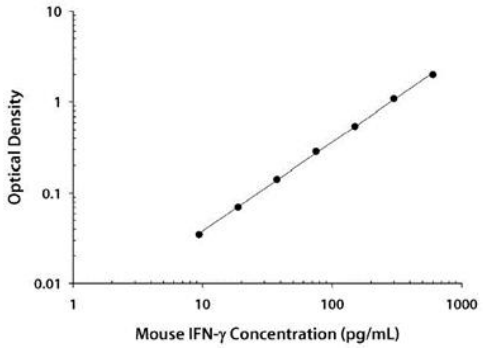
此ELISA法可检测天然及重组小鼠IFN-γ蛋白。将以下因子配制成50 ng/mL的浓度来检测与小鼠IFN-γ的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠IFN-γ对照品中，检测对小鼠IFN-γ的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:	
IL-10	IFN-γ R1
IFN-kappa	IFN-γ R2

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	0.042 0.043	0.042	—
9.4	0.077 0.077	0.077	0.035
18.8	0.113 0.112	0.112	0.070
37.5	0.183 0.186	0.184	0.142
75	0.327 0.329	0.328	0.286
150	0.580 0.594	0.587	0.545
300	1.130 1.124	1.127	1.085
600	2.020 2.042	2.031	1.989

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IFN-γ Microplate	包被小鼠IFN-γ抗体的96孔聚苯乙烯板，8孔×12条	1块板
Mouse IFN-γ Conjugate	酶标检测小鼠IFN-γ抗体	1瓶
Mouse IFN-γ Standard	小鼠IFN-γ标准品（冻干粉），参考瓶身标签进行重溶	2瓶
Diluent（5×）/RD5P	浓缩的稀释液（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/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="6">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>使用时新鲜配制*
标准品-20℃储存，最多 30 天*</td></tr>
<tr><td>稀释液（5×）/RD5P</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的（1×）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。

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 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。样本可能需要用稀释液（1×）稀释。

B. 样本准备工作

小鼠血清样本建议用稀释液（1×）2倍稀释后进行检测，即100 µL血清+100 µL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。

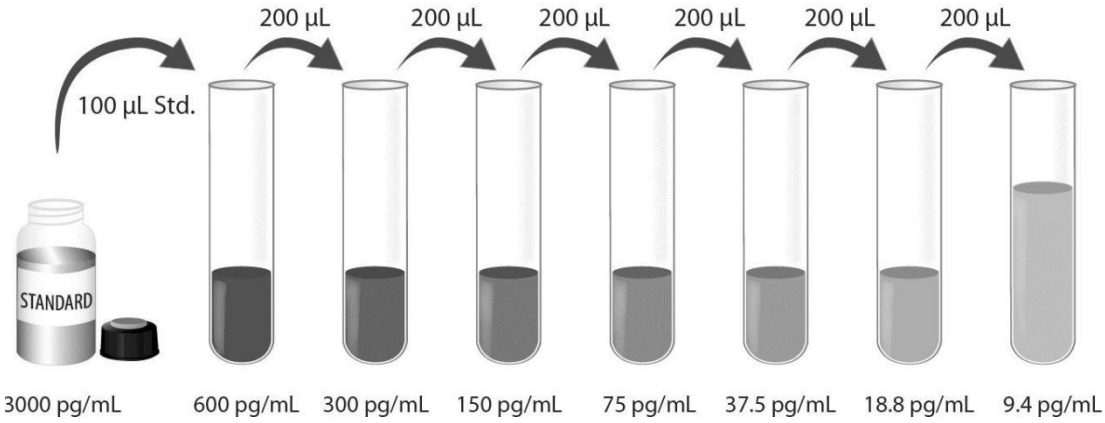
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀， 待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

稀释液（1×）：使用去离子水或蒸馏水稀释配制成稀释液（1×）。

小鼠IFN-γ标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，得到浓度为3000 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。

在600 pg/mL稀释管中加入400 µL稀释液（1×），其余每个稀释管中加入200 µL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。标准品600 pg/mL可用作标准曲线最高点，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，TMB底物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；
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小鼠IFN-γ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IFN-γ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