PRODUCT INFORMATION & MANUAL

Mouse IFN-γ Valukine™ ELISA

VAL607

For the detection of mouse IFN-γ

For research use only. Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 3 months from date of receipt

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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. Monoclonal 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 polyclonal antibody specific for mouse IFN-γ 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 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.**
- 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)	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 in cell culture media was evaluated. The recovery ranged from 89-110% with an average of 96%.

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, six cell culture media samples were 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 ug/mL PHA and 10 ng/mL PMA for 4 days.The cell culture supernate was assayed for mouse IFN-γ and measured 51pg/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.

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:

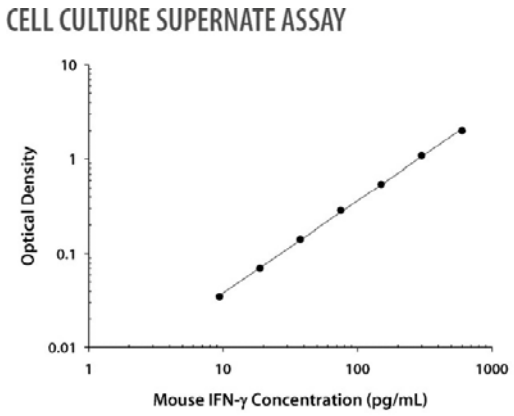
IL-10 IFN-γ R1

IFN-γ R2 IFN-kappa

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 monoclonal antibody against mouse IFN-γ	1 plate
Mouse IFN-γ Conjugate	21mL/vial of a polyclonal antibody against mouse IFN-γ conjugated to horseradish peroxidase with preservatives	1 vial
Mouse IFN-γ Standard	Recombinant mouse IFN-γ in a buffered protein base with preservatives; lyophilized.	2 vial
Calibrator Diluent RD5P(5×)	21 mL/vial of a 5× concentrated buffered protein base with preservatives. For cell culture supernate samples.	1 vial
Wash Buffer Concentrate (25×)	21 mL/vial of a 25× concentrated solution of buffered surfactant with preservatives.	1 vial
Color Reagent A	12.5 mL/vial of stabilized hydrogen peroxide.	1 vial
Color Reagent B	12.5 mL/vial of stabilized chromogen (tetramethylbenzidine)	1 vial
Stop Solution 2	23 mL/vial of diluted hydrochloric acid solution; 0.075M Citric Acid	1 vial
Plate Covers	adhesive strip	3 strips

B. STORAGE

Unopened Kit	Store at 2-8 ℃. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Solution	May be stored for up to 1 month at 2-8 ℃.*
	Stop Solution 2	
	Dilution 1×	
	Conjugate	
	Unmixed Substrate A	
	Unmixed Substrate B	
	Standard	Use a new standard for each assay.
	Microplate Wells	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 ℃.*

* 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 2 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 ℃. Avoid repeated freeze-thaw cycles.

B. REAGENT PREPARATION

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

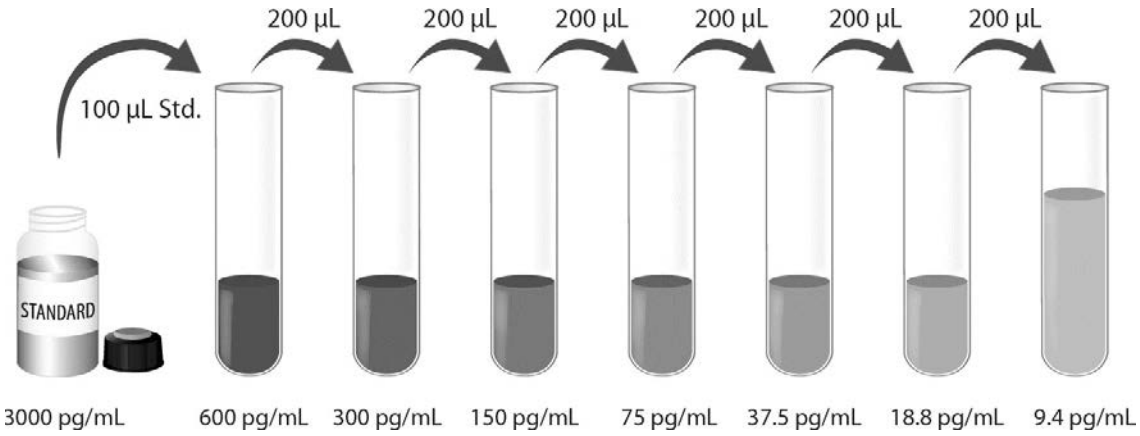
Wash Solution - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Solution Concentrate（25×）into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Substrates 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 Diluent Concentrate 5× into 80 mL of deionized or distilled water to prepare 100 mL of Diluent 1×.

Mouse IFN-γ Standard - Refer to the vial label for reconstitution volume. Reconstitute the Standard with Calibrator Diluent 1×. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

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).



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.
- 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 2 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 2. Wells that are green in color indicate that the Stop solution 2 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 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 Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
9. Add 100 µL of Stop Solution 2 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 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. RERERENCES

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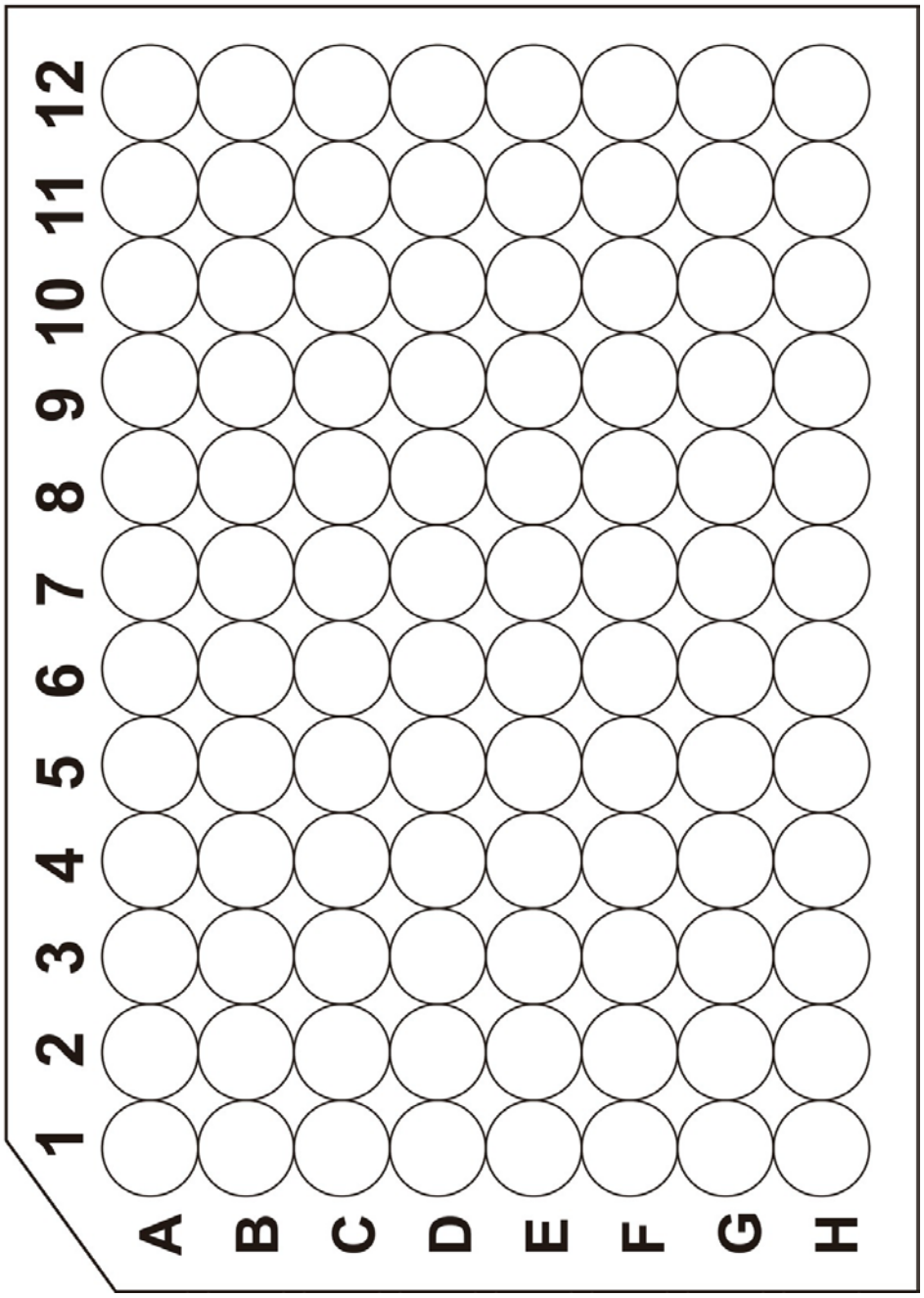
IX. TROUBLESHOOTING

Problem	Probable Cause	Suggestion
No signal	Failure to add all components	Prepare a check-list and add the components in the correct order
Low signal	Not enough supernatant per well	Check the protein concentration. Add more supernatant.
High background	Wells are not washed enough.	Wash plates thoroughly after incubation with detecting.

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

适用于定量测定细胞培养上清液中小鼠 IFN-γ 的含量

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

目录

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 有道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；它知道 B细胞免疫球蛋白的合成、类型交换及分泌。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亚基的活性手提复合体（8，9）。受体复合体中的 IFN-γ RII可层架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 FN-γ 单抗包被于微孔板上，样品和标准品中的IFN-γ 会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗小鼠IFN-γ 多抗，与结合在微孔板上的IFN-γ 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；**
- 试剂盒请在有效期内使用；
- 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，在不同板间分别检测 20 次，以确定板内精确度。

	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不同水平的小鼠 IFN-γ，测定其回收率。回收率范围在89-110%，平均回收率在 96%。

C. 灵敏度

小鼠 IFN-γ 的最低可测值一般小于2.1 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重组小鼠IFN-γ 蛋白所校正。

E. 线 性

6个不同的样本中掺入高浓度的小鼠IFN-γ，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	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-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与 100 mL 的 RPMI1640 的培养基中，细胞培养基还含有 10%胎牛血清、100 U/mL 青霉素, 100 g/mL 硫酸链霉素、10 µg/mL ConA，培养 2 天。取细胞培养上清液测定小鼠 IFN-γ 含量，结果为 3248 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10% 马血清、10 µg/mL PHA和10 ng/mL PMA。取细胞培养上清液测定小鼠IFN-γ 含量，结果为 51 pg/mL。

CTLL-2 细胞以 2×10^4 细胞/mL 植培于 RPMI1640 培养基中，培养 3 天；细胞培养基还含有10%胎牛血清、2mM L-谷氨酸钠、50 mM 巯基乙醇、10 ng/mL 重组小鼠 IL-2；刺激剂为：100 ng/mL 重组小鼠 GM-CSF、2.5 ng/mL LPS。取细胞上清测定小鼠 IFN-γ 含量，未刺激的 CTLL-2 结果为 95 pg/mL; 刺激的 CTLL-2 结果为 306 pg/mL。

刺激细胞，培养 3 天。取细胞培养上清液测定 IL-6 量，结果为 6640 pg/mL。

特异性

此 ELISA 法可检测天然及重组小鼠 IFN-γ 蛋白。将以下因子用稀释剂（1×）配置成 50 ng/mL 的浓度来检测与小鼠 IFN-γ 的交叉反应。将干扰因子掺入中间浓度重组小鼠 IFN-γ 标准中，使其浓度到达 50 ng/mL，以此来检测对小鼠 IFN-γ 的干扰。没有观察到明显的交叉反应或干扰。

重组小鼠蛋白：

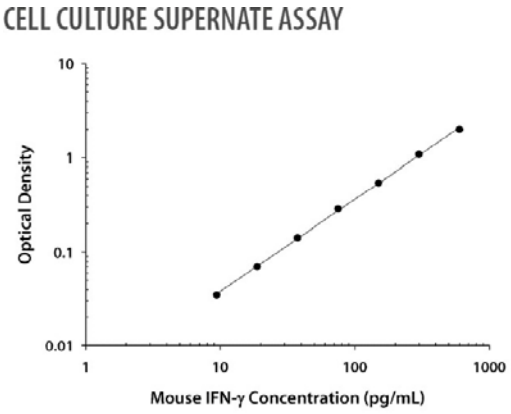
IL-10　　IFN-γ R1

IFN-γ R2　　IFN-kappa

IV. 实验

实验标准

提供的标准曲线数据仅供参考，应根据同次试验所绘标准曲线计算样本含量。



(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
小鼠 IFN-γ Microplate	包被抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
小鼠 IFN-γ Conjugate	酶标检测 IFN-γ 抗体	1 瓶
小鼠 IFN-γ Standard	标准品（冻干）	2 瓶
Calibrator Diluent RD5P（5×）	浓缩标准品稀释缓冲液（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 2	终止液 2，23 mL/瓶	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

未开封试剂盒	2-8℃储存；请在试剂盒有效期内使用	
已打开，稀释或重溶的试剂	洗涤缓冲液（1×）	2-8℃储存，30 天*。
	终止液 2	
	稀释液 1×	
	酶标检测抗体	
	显色剂 A	
	显色剂 B	
	标准品	已重溶的标准品仅限当天使用。
	包被的微孔板条	将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8℃储存，30 天*。

*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2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 ℃电冰箱内，避免反复冻融。样本可能需要用稀释剂（1×）稀释。

B. 检测前准备工作

使用前请将所有试剂放置于室温。

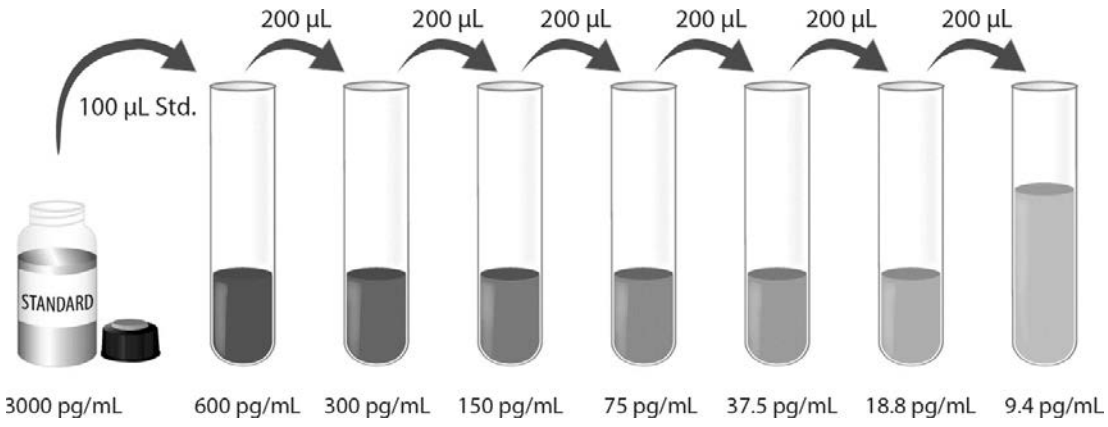
洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完成溶解后再配制洗涤液。可将 20 mL 浓缩洗涤液用蒸馏水或去离子水稀释配置成 500mL 工作浓缩的洗涤液。未用完的放回 4 ℃。

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 分钟准备，仅供当日使用；每孔需 100 µL。

稀释剂（1×）：可将 20mL 浓缩稀释剂用 80mL 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 3000 pg/mL 标准品母液。轻轻震摇至少 5 分钟，其充分溶解。

在 600 pg/mL 稀释管中加入 400 µL 稀释剂（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。没有稀释的标准品母液可用作标准曲线最高点（600 pg/mL），稀释剂（1×）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 2 上板顺序应同显色底物上板顺序一致；加入终止液 2 后，孔内颜色由蓝变黄；若空内有绿色，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 2，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 2 后 30 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：**每一个标准品及样本的复孔校正吸收光值（OD450-OD540/OD570）取平均值，再减去标准曲线零点的 OD 值。利用酶标仪携带的软件，绘制一个 4 参数（4-PL） 线性标准曲线，曲线横坐标为标准曲线点的小鼠 IFN-γ 浓度值，纵坐标为标准曲线点的OD 平均值。通过样本的 OD 值，可从标准曲线上得到样本中小鼠 IFN-γ 的浓度。若样本经过稀释，计算浓度时应乘以稀释倍数。

VIII. 参考文献

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IX. 疑难解答

问题	可能的原因	解决方案
无信号	并未加入所有组分	准备一个检查表并按照正确的顺序添加组件
低信号	每孔内上清不够	检查待测蛋白浓度，可以添加更多的样本
高信号	洗涤不够充分	充分洗板，增加洗涤次数

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