PRODUCT INFORMATION & MANUAL

Mouse IFN-γ Valukine™ ELISA

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

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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.**
- 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)	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 89.0-110.0% 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 74.0-85.3% 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.

Serum - Four Serum samples were evaluated for the presence of 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

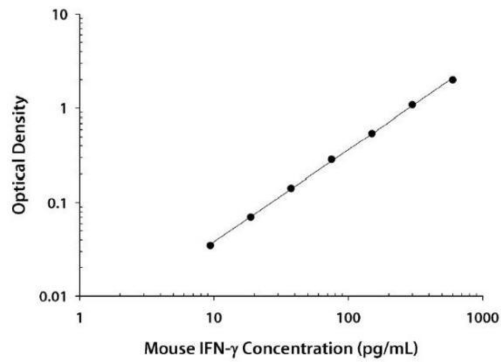
This assay recognizes both natural and recombinant mouse IFN-γ. The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant mouse IFN-γ control were assayed for interference. No significant cross-reactivity 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	0.042	—
	0.043		
9.4	0.077	0.077	0.035
	0.077		
18.8	0.113	0.112	0.070
	0.112		
37.5	0.183	0.184	0.142
	0.186		
75	0.327	0.328	0.286
	0.329		
150	0.580	0.587	0.545
	0.594		
300	1.130	1.127	1.085
	1.124		
600	2.020	2.031	1.989
	2.042		

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	solution 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 vials
Calibrator Diluent (5×)	a 5× concentrated buffered protein base with preservatives	1 vial
Wash Buffer Concentrate (25×)	a 25× concentrated solution of buffered surfactant with preservatives	1 vial
TMB Substrate	TMB ELISA Substrate Solution	1 vial
Stop Solution	diluted hydrochloric acid solution	1 vial
Plate Covers	adhesive strip	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="7">Opened/ Reconstituted Reagents</td><td>Diluted Wash Solution</td><td rowspan="5">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>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Use a new standard 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.

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.*

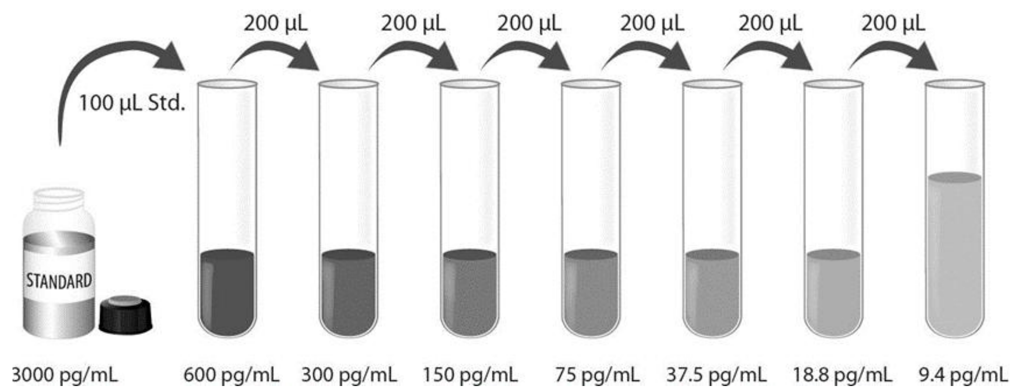
Wash Solution - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

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 IFN-γ Standard - 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 5 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 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 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. 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 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, 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. REFERENCES

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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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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TMB底物溶液，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；
- 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
数量	20	20	20	20	20
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89-110%，平均回收率在96%。

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2.1pg/mL。

最低可测值是根据20个标准曲线零点吸光值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此ELISA试剂盒经R&D Systems生产的大肠杆菌表达的高纯度重组小鼠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100mL的RPMI1640的培养基中，细胞培养基还含有10%胎牛血清、2mM L-谷氨酸钠、100U/mL青霉素, 100g/mL硫酸链霉素、10μg/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3248pg/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100mL的DMEM含有10%马血清、10μg/mL PHA和10ng/mL PMA。取细胞培养上清液测定小鼠IFN-γ含量，结果为51pg/mL。

CTLL-2细胞以5×10^{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、50mM巯基乙醇、10ng/mL重组小鼠IL-2；刺激剂为：100ng/mL重组小鼠GM-CSF、2.5ng/mL LPS。取细胞上清测定小鼠IFN-γ含量， 未刺激的CTLL-2结果为95pg/mL; 刺激的CTLL-2结果为306pg/mL。

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。4份样本的检测值在28.6-48.3pg/mL之间，平均值为39.0pg/mL。

G. 特异性

此ELISA法可检测天然及重组小鼠IFN-γ蛋白。将以下因子用稀释剂（1×）配置成50ng/mL的浓度来检测与小鼠IFN-γ的交叉反应。将50ng/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。

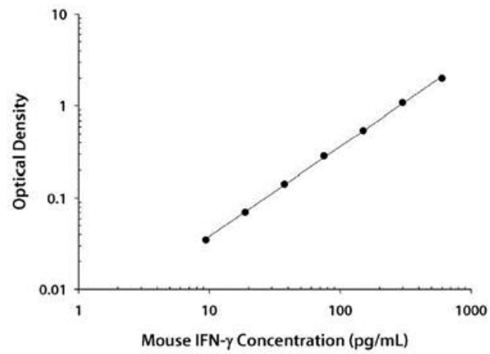
重组小鼠蛋白	
IL-10	IFN-γ R1
IFN-kappa	IFN-γ R2

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。

CELL CULTURE SUPERNATE ASSAY



(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IFN-γ抗体	1瓶
Mouse IFN-γ Standard	标准品（冻干）	2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
TMB Substrate	TMB底物溶液	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="5">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>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>已重溶的标准品仅限当天使用。</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450nm检测波长的吸收值及540nm或570nm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；
- 500mL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100μL血清+100μL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。

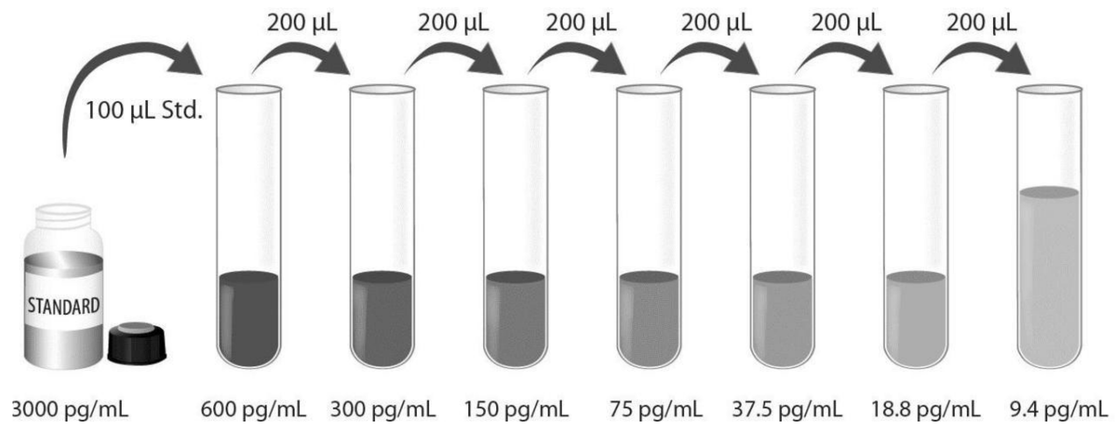
洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20mL浓缩洗涤液用蒸馏水或去离子水稀释配置成500mL工作浓度的洗涤液。未用完的放回4℃。

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100mL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3000pg/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。

在600pg/mL稀释管中加入400μL稀释剂（1×），其余每个稀释管中加入200μL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。标准品600pg/mL可用作标准曲线最高点，稀释剂（1×）可用作标准曲线零点（0pg/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；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀请充分混合。

VII.操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 在每个微孔中加入50μL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50μL。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400μL，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100μL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100μL 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100μL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10分钟内，使用酶标仪测量450nm的吸光度值，设定540nm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

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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