



PRODUCT INFORMATION & MANUAL

Primate IFN- γ Valukine™ ELISA

VAL1001

For the quantitative determination of natural and recombinant
Primate 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

Version202210.1

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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). It plays key roles in host defense by exerting antiviral, antiproliferative and immunoregulatory activities (2-5). 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 potentiates the secretion of immunoglobulins by B cells and directs the synthesis of IgG. IFN- γ also influences T-helper cell phenotype development by inhibiting Th2 differentiation and stimulating Th1 development (2-5).

IFN- γ is produced by a number of cell types, including dendritic epidermal/gd T cells (6), keratinocytes (7), peripheral blood $\gamma\delta$ T cells (8), mast cells (9), neurons (10), CD8+ T cells (11), macrophages (12), B cells (13), neutrophils (14), NK cells (15), CD4+ T cells (16) and testicular spermatids (17). The production of IFN- γ is upregulated synergistically by IL-12, IL-18, and IL-23 (18-21). Rhesus monkey IFN- γ cDNA encodes a 165 amino acid (aa) residue precursor protein that contains a 20 aa signal sequence that is cleaved to generate a 145 aa residue mature monkey IFN- γ (22). Rhesus monkey IFN- γ is presumably a noncovalent homodimer (3). In the mature segment, it shares 94%, 88%, 100%, 100%, 98%, and 94% aa sequence identity with human (23, 24), squirrel monkey (25), pigtailed macaque (22), cynomolgus monkey (26), sooty mangabey (22), and gorilla (27) IFN- γ , respectively (22). By contrast, mature human IFN- γ shares 94%, 90%, 94%, 94%, 93%, and 100% aa sequence identity with rhesus monkey (22), squirrel monkey (25), pigtailed macaque (22), cynomolgus monkey (26), sooty mangabey (22), and gorilla (27) IFN- γ , respectively.

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for primate IFN- γ has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN- γ present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for primate IFN- γ is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate solution is added to the wells and color develops in proportion to the amount of primate 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 supernates and primate serum.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent (1 \times) 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)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
Mean (pg/mL)	32.5	240.1	483.3	32.7	242.1	491.7
Standard Deviation	1.2	5.4	22.2	1.1	8.8	24.6
CV%	3.5	2.2	4.6	3.4	3.6	5.0

B. RECOVERY

The recovery of primate IFN- γ spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 83.1-106.2% with an average of 94.5%.

The recovery of primate IFN- γ spiked to different levels throughout the range of the assay in primate serum was evaluated. The recovery ranged from 82.6-101.1% with an average of 93.2%.

C. SENSITIVITY

The minimum detectable dose (MDD) of Primate IFN- γ is typically less than 0.52 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 a highly purified *E. coli* -expressed recombinant primate 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 primate IFN- γ and diluted with Calibrator Diluent (1 \times) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	97.0	83.9-110.6
1:4	100.2	89.0-113.7
1:8	105.0	87.8-119.7
1:16	99.1	78.3-120.1

F. SAMPLE VALUES

Serum - Six primate serum samples were evaluated for the presence of primate IFN- γ in this assay. All samples measured below the lowest standard, 15.6 pg/mL.

G. SPECIFICITY

The following factors were tested in the assay at 50 ng/mL and no cross-reactivity was identified.

Recombinant Murine IFN- γ

Recombinant Canine IFN- γ

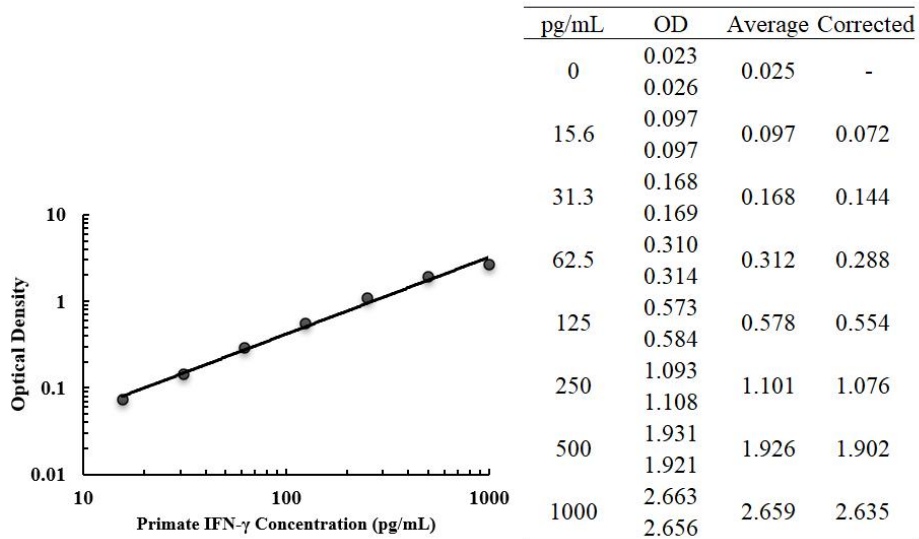
Recombinant Equine IFN- γ

Recombinant Rat IFN- γ

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Primate IFN- γ Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a rat antibody against primate IFN- γ .	1 plate
Primate IFN- γ Standard	Recombinant primate IFN- γ in a buffered base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Primate IFN- γ Detection Antibody	Biotinylated primate IFN- γ antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent Concentrate (2 \times)	Buffered diluent used to dilute standard and samples.	1 vial
Reagent Diluent Concentrate (10 \times)	Buffered protein base used to dilute Detection Antibody and Streptavidin-HRP.	1 vial
Streptavidin-HRP A (200 \times)	200 \times Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25 \times)	A 25 \times concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB Substrate Solution.	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP A	May be stored for up to 1 month at 2-8 °C.*
	Diluted Wash Solution	
	TMB Substrate	
	Stop Solution	
	Standard	Prepare fresh for each assay. Standards may be stored for up to 1 month at -20 °C.*
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	Reagent Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.
	Calibrator Diluent Concentrate (2×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.
Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*	

* 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.
- ◆ Squirrt bottle, manifold dispenser, or automated microplate washer.
- ◆ 500 mL graduated cylinder.

D. PRECAUTION

- ◆ Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- ◆ The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples require dilution with Calibrator Diluent (1 \times).

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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples require dilution with Calibrator Diluent (1 \times).

B. SAMPLE PREPARATION

Cell culture supernates and serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 μL of sample + 100 μL of Calibrator Diluent (1 \times).

C. REAGENT PREPARATION

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

Wash Buffer (1 \times)- 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 \times) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 \times).

Reagent Diluent (1 \times) - Use deionized or distilled water to prepare Reagent Diluent (1 \times).

Calibrator Diluent (1 \times) - Use deionized or distilled water to prepare Calibrator Diluent (1 \times).

Detection Antibody- **Centrifuge briefly before opening. Reconstitution Volume refer to vial label with Reagent Diluent (1 \times)**. Aliquot and store if needed. Allow the detection antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Dilute stock solution in Reagent Diluent (1 \times) to the working concentration of 250 ng/mL. Prepare at least 15 minutes prior to use.

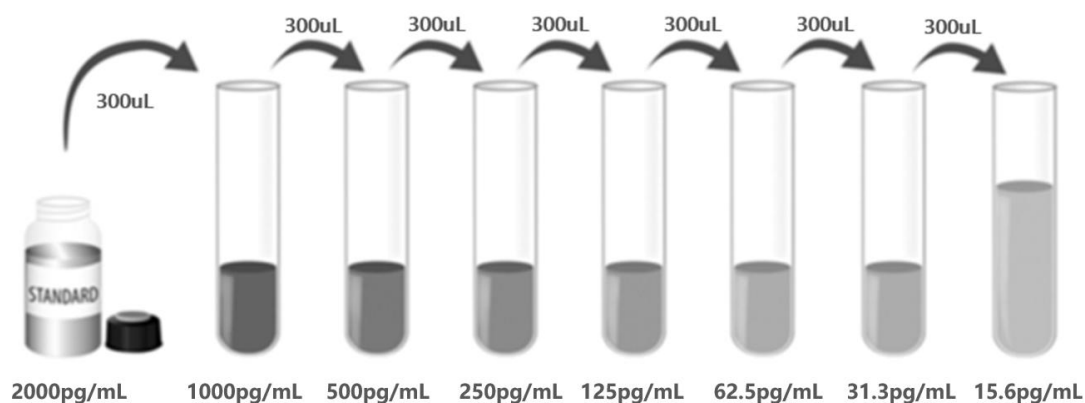
Streptavidin-HRP A (1 \times) - **Centrifuge briefly before opening.** Dilute to the working concentration specified on the vial label using Reagent Diluent (1 \times).

Primate IFN- γ Standard - **Centrifuge briefly before opening. Refer to the vial**

label for the reconstitution volume*. This reconstitution produces a stock solution of 2000 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 300 μ L of the appropriate Calibrator Diluent (1 \times) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The Calibrator Diluent (1 \times) 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 100 μ L of standard, or prepared 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. (Samples may require dilution. See Sample Preparation section.)
4. 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 μ L) 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.
5. Add 100 μ L of the Detection Antibody diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate **2 hours at room temperature**. **Avoid placing the plate in direct light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μ L of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for **30 minutes at room temperature**. **Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μ L of TMB Substrate to each well. Incubate for **20 minutes at room temperature**. **Avoid placing the plate in direct light.**
10. Add 50 μ L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. 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.

12. CALCULATION OF RESULTS

Average the duplicate readings for each standard, sample and subtract the average zero standard optical density (O.D.). 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 primate 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.

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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																	
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11																	
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	A	B	C	D	E	F	G	H									



产品信息及操作手册

灵长类 IFN- γ Valukine™ ELISA 试剂盒

目录号: VAL1001

适用于定量检测天然和重组灵长类 IFN- γ 的浓度

科研专用, 不可用于临床诊断

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有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

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I. 背景

干扰素- γ (IFN- γ , 也称为II型干扰素) 是一种重要的免疫调节细胞因子, 最初是通过其抗病毒活性发现的 (1)。它通过发挥抗病毒、抗增殖和免疫调节活性在宿主防御中发挥关键作用 (2-5)。在许多细胞类型上, IFN- γ 诱导细胞因子的产生并上调各种膜蛋白的表达, 包括I类和II类MHC抗原、Fc受体、白细胞粘附分子和B7家族抗原。IFN- γ 是巨噬细胞效应功能的有效激活剂。它增强B细胞分泌免疫球蛋白并指导IgG的合成。IFN- γ 还通过抑制Th2分化和刺激Th1发育来影响T辅助细胞表型发育 (2-5)。

IFN- γ 由多种细胞类型产生, 包括树突状表皮/gd T细胞 (6)、角质形成细胞 (7)、外周血 $\gamma\delta$ T细胞 (8)、肥大细胞 (9)、神经元 (10)、CD8⁺ T细胞 (11)、巨噬细胞 (12)、B细胞 (13)、中性粒细胞 (14)、NK 细胞 (15)、CD4⁺ T细胞(16)和睾丸精子细胞 (17)。IL-12、IL-18和IL-23协同上调IFN- γ 的产生 (18-21)。恒河猴IFN- γ cDNA编码一个165个氨基酸(aa) 残基的前体蛋白, 该前体蛋白包含一个20个aa的信号序列, 该信号序列被切割以产生一个145个aa残基的成熟猴IFN- γ (22)。恒河猴IFN- γ 可能是一种非共价同源二聚体 (3)。在成熟阶段, 它与人类 (23, 24)、松鼠猴 (25)、辫子猕猴 (22)、食蟹猴(26)、白眉猴 (22) 和大猩猩 (27) 的IFN- γ , 分别具有94%、88%、100%、100%、98%和94%的aa序列同源性。相比之下, 成熟的人IFN- γ 与恒河猴 (22)、松鼠猴 (25)、辫子猕猴 (22)、食蟹猴 (26)、白眉猴 (22) 和大猩猩 (27) 的IFN- γ , 分别具有94%、90%、94%、94%、93%和100%的aa序列同源性。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗灵长类IFN- γ 抗体包被于微孔板上，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；接着加入生物素化的抗灵长类IFN- γ 检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（Streptavidin-HRP）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灵长类血清样本；
- ◆ 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，应将样本用标准品稀释液（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）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测20次，以确定板间精确度。

样本	板内精确度			板间精确度		
	1	2	3	1	2	3
平均值 (pg/mL)	32.5	240.1	483.3	32.7	242.1	491.7
标准差	1.2	5.4	22.2	1.1	8.8	24.6
CV%	3.5	2.2	4.6	3.4	3.6	5.0

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灵长类IFN- γ ，测定其回收率。回收率范围在83.1-106.2%，平均回收率在94.5%。

在灵长类血清样本中掺入检测范围内不同水平的灵长类IFN- γ ，测定其回收率。回收率范围在82.6-101.1%，平均回收率在93.2%。

C. 灵敏度

灵长类IFN- γ 的最低可测剂量（MDD）一般小于0.52 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浓度。

D. 校正

此ELISA试剂盒经由R&D Systems生产的*E. coli*表达的高纯度重组灵长类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灵长类IFN- γ ，然后用标准品稀释液（1 \times ）将样本稀

释到检测范围内，测定其线性。

稀释倍数	平均值 (%)	范围 (%)
1:2	97.0	83.9-110.6
1:4	100.2	89.0-113.7
1:8	105.0	87.8-119.7
1:16	99.1	78.3-120.1

F. 样本预值

血清样本 - 使用本试剂盒检测了6份灵长类血清样本中IFN- γ 的水平。样本的检测值均低于最低标准品，15.6pg/mL。

G. 特异性

将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应。

重组小鼠IFN- γ

重组犬IFN- γ

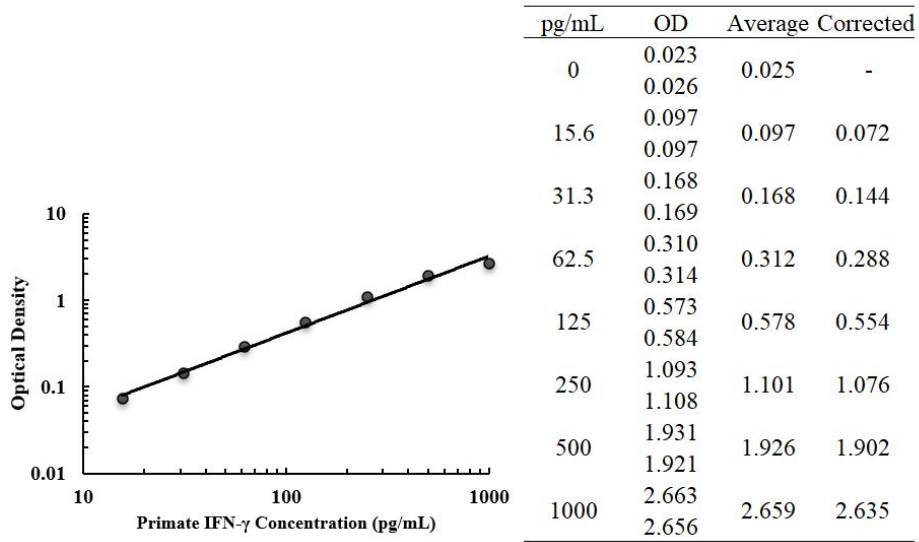
重组马IFN- γ

重组大鼠IFN- γ

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。



V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Primate IFN- γ Microplate	包被大鼠抗灵长类 IFN- γ 抗体的 96 孔聚苯乙烯板, 8 孔 \times 12 条	1 块板
Primate IFN- γ Standard	标准品 (冻干粉), 参考瓶身标签进行重溶	2 瓶
Primate IFN- γ Detection Antibody	生物素化的 IFN- γ 检测抗体, 冻干粉, 参考瓶身标签进行重溶	1 瓶
Calibrator Diluent Concentrate (2 \times)	浓缩的标准品稀释液 (2 \times) 用于稀释标准品和样本。	1 瓶
Reagent Diluent Concentrate (10 \times)	浓缩的试剂稀释液 (10 \times) 用于稀释检测抗体和 Streptavidin-HRP A。	1 瓶
Streptavidin-HRP A (200 \times)	200 \times 浓缩的链霉亲和素标记的 HRP	1 瓶
Wash Buffer Concentrate (25 \times)	浓缩洗涤缓冲液 (25 \times)	1 瓶
TMB Substrate	TMB 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

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	链霉亲和素-HRP A	2-8℃储存，最多 30 天*
	洗涤缓冲液（1×）	
	TMB 底物溶液	
	终止液	
	标准品	使用时新鲜配制* 标准品-20℃储存，最多 30 天*
	检测抗体	分装， -20℃储存，最多 30 天*
	试剂稀释液（10×）	2-8℃储存，最多 30 天* 请每次使用新鲜配制的 1×试剂稀释液
	标准品稀释液（2×）	2-8℃储存，最多 30 天* 请每次使用新鲜配制的 1×标准品稀释液
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，最多 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

- ◆ 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- ◆ 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的保护。使用后请彻底洗手。

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。样本需要用标准品稀释液（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。样本需要用标准品稀释液（1×）稀释。

B. 样本准备工作

细胞上清、血清样本需要用标准品稀释液（1×）2倍稀释后进行检测，例如：100 μL样本+100 μL标准品稀释液（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（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浓缩洗涤液用蒸馏水或去离子水稀释配制成500mL工作浓度的洗涤液（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

标准品稀释液（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液（1×）。

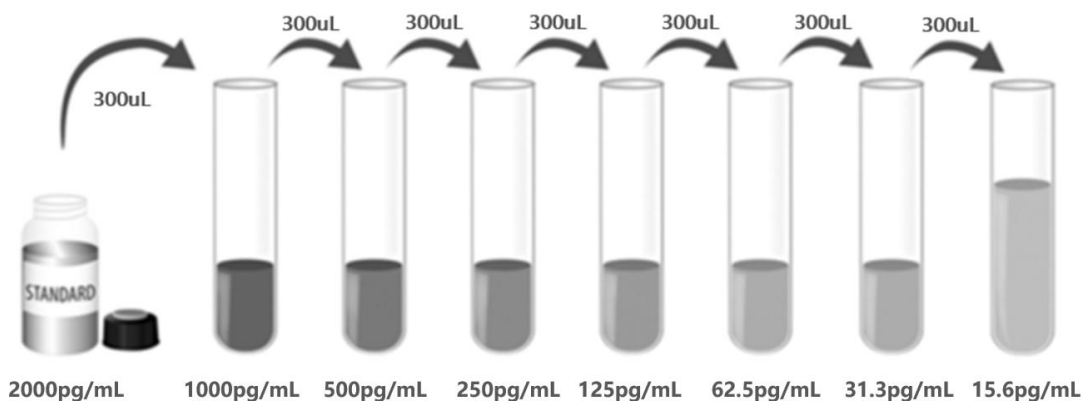
检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶，轻轻震荡至少15分钟，使其充分溶解。再用试剂稀释液（1×）稀释至工作浓度250 ng/mL，至少在使用前15分钟准备。

链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

灵长类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2000 pg/mL标准品母液。轻轻震荡至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300 μL标准品稀释液（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。1000 pg/mL管作标准曲线最高点，标准品稀释液（1×）可用作标准品零点（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，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. 分别将不同浓度标准品、实验样本加入相应孔中，每孔100 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；（样本需要稀释，详情参见样本制备部分。）
4. 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；
5. 在每个微孔内加入100 μL 配制好的检测抗体工作液。用封板膜封住反应孔，**室温孵育2小时，注意避光**；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 μL 稀释好的链霉亲和素- HRP A 工作液。用封板膜封住反应孔，**室温孵育30分钟，注意避光**；
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 μL TMB底物溶液，**室温孵育20分钟，注意避光**；
10. 在每个微孔内加入50 μL 终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色；
11. 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。如果没有使用双波长校正，结果准确度可能会受影响；
12. **计算结果**：将每个标准品和样品的校正吸光度值（ $\text{OD}_{450}-\text{OD}_{540}/\text{OD}_{570}$ ），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。通过样本的OD值，可从标准曲线上得到样本中IFN- γ 的浓度。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

