



PRODUCT INFORMATION & MANUAL

Human IFN- γ Valukine™ ELISA

VAL104

For the quantitative determination of natural and
recombinant human IFN- γ 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 201911.3

TABLE OF CONTENTS

I. BACKGROUND.....	2
II. OVERVIEW.....	3
III. ADVANTAGES.....	4
IV. EXPERIMENT.....	6
V. KIT COMPONENTS AND STORAGE.....	7
VI. PREPARATION.....	9
VII. ASSAY PROCEDURE.....	11
VIII. REFERENCES.....	12

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 anti-viral, anti-proliferative, 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 human IFN- γ shares 90% amino acid (aa) sequence identity with rhesus macaque IFN- γ , 59%-64% with bovine, canine, equine, feline and porcine IFN- γ , and 37%-43% aa identity with cotton rat, mouse, and rat 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 a functional receptor complex of two and two 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 β -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/ $\gamma\delta$ T cells (11), keratinocytes (12), peripheral blood $\gamma\delta$ 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. A polyclonal antibody specific for 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, an enzyme-linked polyclonal antibody specific for 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 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 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.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	47.8	148	321	61.5	164	342
Standard Deviation	3.67	7.57	14.2	5.55	10.4	28.4
CV%	7.7	5.1	4.4	9.0	6.4	8.3

B. RECOVERY

The recovery of human IFN- γ spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 90-116% with an average of 103%.

The recovery of human IFN- γ spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 88.8-100.5% with an average of 96.3%.

C. SENSITIVITY

The minimum detectable dose (MDD) of IFN- γ is typically less than 7.8 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 human IFN- γ produced at R&D Systems. The NIBSC 1st British Standard for human leukocyte IFN- γ (82/587), which was intended as a potency standard, was evaluated in this kit.

The dose response curve of the NIBSC Standard (82/587) parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human IFN- γ kit to approximate NIBSC 82/587 units, use the equation below.

NIBSC (82/587) approximate value (IU/mL) = 0.010 × Valukine Human IFN- γ value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of IFN- γ and diluted with Diluent 1 \times to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	107	96 – 109
1:4	111	107 – 115
1:8	111	104 – 121
1:16	106	91 – 114

F. SAMPLE VALUES

Cell Culture Supernates -Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernates was removed on days 1 and 5 and assayed for levels of natural IFN- γ .

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	453	2651
Stimulated	8360	7851

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

G. SPECIFICITY

This assay recognizes both natural and recombinant human 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 rhIFN- γ control were assayed for interference. No significant cross-reactivity or interference was observed.

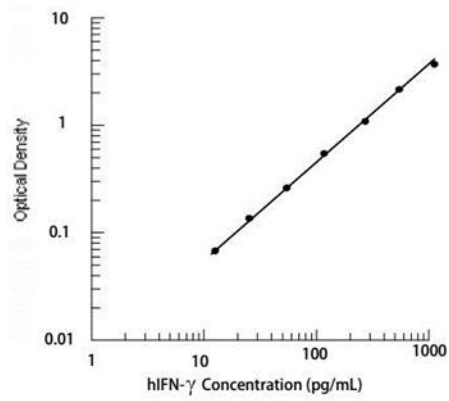
Recombinant human	Recombinant mouse	Recombinant rat
IL-1 α	IFN- γ	IFN- γ
IL-1 β	IL-1 α	
IL-1 α	IL-1 β	
IL-6	IL-6	
SCF		

Recombinant rhesus macaque IFN- γ was found to cross-react approximately 20%.

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	OD	Average	Corrected
0	0.085 0.087 0.161	0.086	—
15.6	0.170 0.228	0.166	0.080
31.3	0.232 0.343	0.230	0.144
62.5	0.375 0.574	0.359	0.273
125	0.624 1.013	0.599	0.513
250	1.069 1.762	1.041	0.955
500	1.914 2.969	1.838	1.752
1000	3.000	2.985	2.899

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
IFN- γ Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse polyclonal antibody against human IFN- γ	1 plate
IFN- γ Conjugate	a solution of polyclonal antibody against IFN- γ conjugated to horseradish peroxidase	1 vial
IFN- γ Standard	recombinant human IFN- γ in a buffered protein base; lyophilized	1 vial
Calibrator Diluent(5 \times)	a 5 \times concentrated buffered protein base	1 vial
Wash Buffer Concentrate(25 \times)	a 25 \times 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	2 N sulfuric acid	1 vial
Plate Covers	adhesive strip	3 strips

B. STORAGE

Unopened Kit	Store at 2-8°C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Solution	May be stored for up to 1 month at 2-8°C.*
	Stop Solution	
	Diluent 1×	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	Aliquot and store for up to 1 month at <-20°C. in a manual defrost freezer. * Avoid repeated freeze-thaw cycles.
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.
- ◆ 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with 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.

B. SAMPLE PREPARATION

Serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 μL of sample + 100 μL of Diluent (1 \times).

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

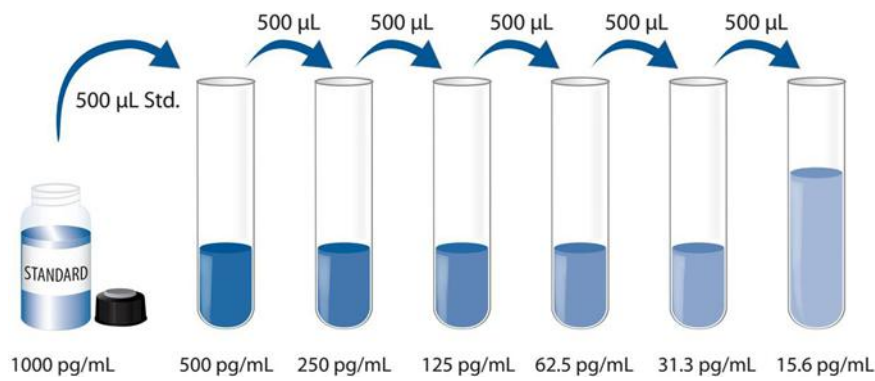
Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μL of the resultant mixture is required per well.

Diluent 1 \times - Add 20 mL of Calibrator Diluent 5 \times into 80 mL of deionized or distilled water to prepare 100 mL of Diluent 1 \times .

IFN- γ Standard - Refer to the vial label for reconstitution volume for reconstitution*. This reconstitution produces a stock solution of 1000 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.

Use polypropylene tubes. Pipette 500 μL of 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 Diluent 1 \times serves as the zero standard (0pg/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 100 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.
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 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.
5. Add 200 μL of IFN- γ Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
8. Add 50 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.
9. 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.
10. **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

1. Billiau, A. and P. Matthys (2009) Cytokine Growth Factor Rev. 20:97.
2. Wheelock, E.F. (1965) Science 146:310.
3. Schoenborn, J.R & C.B. Wilson (2007) Adv. Immunol. 96:41.
4. Pestka, S. et al. (2004) Immunol. Rev. 205:8.
5. Kelchtermans, H. et al. (2008) Trends Immunol. 29:479.
6. McLaren, J.E. and D.P. Ramji (20 09) Cytokine Growth Factor Rev. 20:125.
7. Gray, P.W. and D.V. Goeddel (1982) Nature 298:859.
8. Bach, E.E. et al. (1997) Annu. Rev. Immunol. 15:563.
9. Marsters, S.A. et al. (1995) Proc. Natl. Acad. Sci. 92:5401.
10. Krause, C.D. et al. (2000) J. Biol. Chem. 275:22995.
11. Sugaya, M. et al. (1999) J. Invest. Dermatol. 113:350.
12. Howie, S.E.M. et al. (1996) J. Invest. Dermatol. 106:1218.
13. Battistini, L. et al. (1997) J. Immunol. 159:3723.
14. Gupta, A.A. et al. (1996) J. Immunol. 157:2123.
15. Neumann, H. et al. (1997) J. Exp. Med. 186:2023.
16. Hoiden, I. & G. Moller (1996) Scand. J. Immunol. 44:501.
17. Puddu, P. et al. (1997) J. Immunol. 159:3490.
18. Yoshimoto, T. et al. (1997) Proc. Natl. Acad. Sci. USA 94:3948.
19. Yeaman, G.R. et al. (1998) J. Immunol. 160:5145.
20. Asea, A. et al. (1996) Clin. Exp. Immunol. 105:376.
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.

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

人 IFN- γ Valukine™ ELISA 试剂盒

目录号: VAL104

适用于定量检测天然和重组和重组人干扰素 gamma (IFN- γ) 的含量

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.....	16
II. 概述.....	17
III. 优势.....	18
IV. 实验标准.....	20
V. 试剂盒组成及储存.....	21
VI. 实验前准备.....	23
VII. 操作步骤.....	25
VIII. 参考文献.....	26

I. 背景

γ 干扰素（又称为II型干扰素）是一个重要的能行使免疫调节功能的细胞因子。它因其抗病毒活性而被发现（1, 2）。 γ 干扰素通过抗病毒、抗增殖和免疫调节功能在宿主防御中扮演关键角色（3, 4）。在多种类型的细胞中， γ 干扰素可诱导细胞因子的产生和上调不同的膜蛋白的表达，包括I/II型主要组织相容性复合体（MHC-I, MHC-II）、Fc受体、白细胞粘附分子、B7家族抗原。 γ 干扰素能有效活化巨噬细胞、指导B细胞免疫球蛋白的合成、类型转化和分泌。 γ 干扰素也能通过抑制Th2的分化和刺激Th1的生长而影响T辅助细胞亚型的发育。 γ 干扰素在自身免疫和动脉粥样硬化症等一些炎症性疾病过程中起着重要作用（5, 6）。

生物活性的 γ 干扰素是一个非共价结合的二聚体，其分子量约为20-25 KD，有不同程度的糖基化（7）。成熟的人 γ 干扰素与恒河猴的 γ 干扰素氨基酸序列同源性接近90%，与牛、犬、马、猫和猪的 γ 干扰素有59-64%的同源性，与棉鼠、小鼠和大鼠的 γ 干扰素同源性为37-43%。干扰素二聚体先与跨膜受体IFN- γ RI（ α 亚基）结合，再与IFN- γ RII（ β 亚基）结合形成一包含了两个 α 亚基和两个 β 亚基的功能性复合体（8, 9）；其中复合受体中的IFN- γ RII可以增加配体的亲和力以及信号转导效率（9, 10）。尽管 γ 亚基在很多类型细胞上有组成型表达，但 β 亚基细胞表达的调节受体 γ 干扰素反应状态的影响，并被严格调控。

γ 干扰素在一系列炎症条件下可由许多的细胞分泌，包括树突状表皮 $\gamma\delta$ T细胞（11）、角质化细胞（12）、外周血 $\gamma\delta$ 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）等等。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗人 IFN- γ 多抗包被于微孔板上，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；加入辣根过氧化物酶标记的抗人 IFN- γ 多抗，未结合的抗体被洗去；加入底物溶液（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人血清样本；
- ◆ 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；若细胞培养上清液样本需分步稀释，除最后一步用稀释剂稀释外，其它中间稀释可采用细胞培养基；
- ◆ 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，在不同板间分别检测 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)	47.8	148	321	61.5	164	342
标准差	3.67	7.57	14.2	5.55	10.4	28.4
CV%	7.7	5.1	4.4	9.0	6.4	8.3

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人IFN- γ ，测定其回收率。回收率范围在 90-116%，平均回收率在 103%。

在人血清样本中掺入检测范围内不同水平的人IFN- γ ，测定其回收率。回收率范围在88.8-100.5%，平均回收率在96.3%。

C. 灵敏度

人IFN- γ 的最低可测值一般小于 7.8 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 试剂盒是针对R&D Systems 生产的大肠杆菌表达的高纯度重组人IFN- γ 进行校准的。NIBSC IFN- γ 第1英国标准品(82/587)作为效价标准，在本试剂盒中进行了评估。

NIBSC标准品(82/587)的剂量反应曲线与Valukine 标准曲线平行。若要将使用Valukine Human IFN- γ kit获得的样本值转换为NIBSC 82/587的近似单位，请使用以下公式：

NIBSC (82/587) approximate value (IU/mL) = 0.010 × Valukine Human IFN- γ value (pg/mL)

E. 线性

不同的样本中含有或掺入高浓度的人IFN- γ ，然后用稀释剂（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/期待值 (%)	范围 (%)
1:2	107	96 - 109
1:4	111	107 - 115
1:8	111	104 - 121
1:16	106	91 - 114

F. 样本预值

细胞上清样本 - 人的外周血单核细胞（ 1×10^6 细胞/mL）培养于含有 10%胎牛血清的 RPMI1640 培养基中，细胞培养基还含有 2 mM L-谷氨酰胺、50 μ M β -巯基乙醇、100 U/mL 青霉素，100 μ g/mL 链霉素。部分另加 10 μ g/mL PHA 刺激细胞，在第一天和第五天，取少量未经刺激和刺激后的细胞上清液测定 IFN- γ 含量，结果见下表。

条件	第一天 (pg/mL)	第五天 (pg/mL)
未刺激	453	2651
刺激	8360	7851

血清样本 - 使用本试剂盒检测了4份人血清样本中IFN- γ 的水平。4份样本的检测值均低于最低标准品，15.6pg/mL。

G. 特异性

此ELISA 法可检测天然及重组人 IFN- γ 蛋白。将以下因子用稀释剂（1 \times ）配置成 50 ng/mL 的浓度来检测与人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人IFN- γ 对照品中，来检测对人IFN- γ 的干扰。没有观察到明显的交叉反应或干扰。

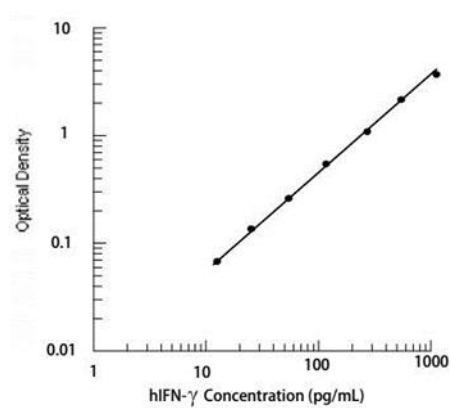
重组人蛋白	重组小鼠蛋白	重组大鼠蛋白
IL-1 α	IFN- γ	IFN- γ
IL-1 β	IL-1 α	
IL-1 α	IL-1 β	
IL-6	IL-6	
SCF		

与重组恒河猴 IFN- γ 有大约 20%的交叉反应。

IV. 实验标准

标准曲线实例

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	OD	Average	Corrected
0	0.085 0.087 0.161	0.086	—
15.6	0.170 0.228	0.166	0.080
31.3	0.232 0.343	0.230	0.144
62.5	0.375 0.574	0.359	0.273
125	0.624 1.013	0.599	0.513
250	1.069 1.762	1.041	0.955
500	1.914 2.969	1.838	1.752
1000	3.000	2.985	2.899

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
IFN- γ Microplate	包被抗体的 96 孔聚苯乙烯板, 8 孔 \times 12 条	1 块板
IFN- γ Conjugate	酶标检测IFN- γ 抗体	1 瓶
IFN- γ Standard	标准品 (冻干粉)	1 瓶
Calibrator Diluent (5 \times)	浓缩稀释剂 (5 \times)	1 瓶
Wash Buffer Concentrate (25 \times)	浓缩洗涤缓冲液 (25 \times)	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

B. 试剂盒储存

未开封试剂盒	2-8 $^{\circ}$ C 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 \times)	2-8 $^{\circ}$ C 储存, 最多 30 天*
	终止液	
	稀释剂 (1 \times)	
	酶标检测抗体	
	显色剂 A	
	显色剂 B	
	标准品	分装, -20 $^{\circ}$ C 储存最多 30 天*; 避免反复冻融。
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 $^{\circ}$ C 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
- ◆ 聚丙烯试管
- ◆ 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°C 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 $1000 \times g$ 离心15分钟。吸取血清样本之后即刻用于检测, 或者分装, -20°C 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 $100 \mu\text{L}$ 血清+ $100 \mu\text{L}$ 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°C 。

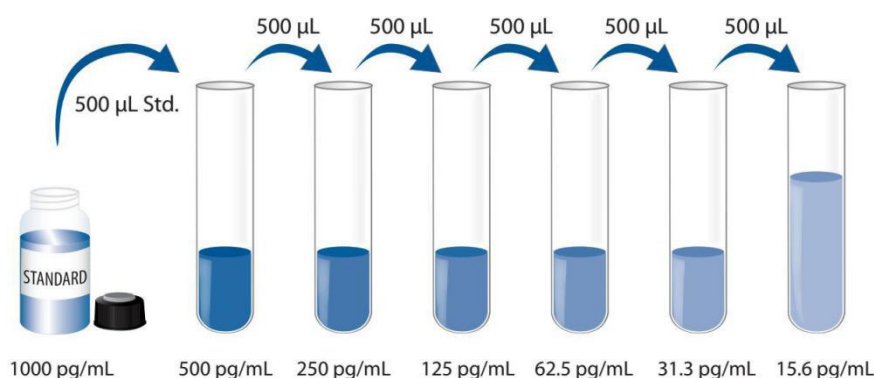
显色剂: 按当次试验所需要用量将显色剂 A 和显色剂B 等体积混合, 避光; 在使用前15分钟内准备, 仅供当日使用; 每孔需 $200 \mu\text{L}$ 。

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: 依照标准品标签上注明的重溶体积重溶冻干标准品, 得到浓度为 1000 pg/mL 标准品母液。轻轻震荡至少15分钟, 其充分溶解。

*如有疑问, 请咨询我们的技术支持。

使用聚丙烯试管作为标准品稀释管。每个稀释管中加入 $500 \mu\text{L}$ 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(1000 pg/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. 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 100 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；
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 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 200 μL 酶标检测抗体。用封板胶纸封住反应孔，室温孵育 2 小时；
6. 重复第 4 步洗板操作；
7. 在每个微孔内加入 200 μL 显色底物，室温孵育 30 分钟。**注意避光；**
8. 在每个微孔内加入 50 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；
9. 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 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；
10. **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。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. Billiau, A. and P. Matthys (2009) Cytokine Growth Factor Rev. 20:97.
2. Wheelock, E.F. (1965) Science 146:310.
3. Schoenborn, J.R & C.B. Wilson (2007) Adv. Immunol. 96:41.
4. Pestka, S. et al. (2004) Immunol. Rev. 205:8.
5. Kelchtermans, H. et al. (2008) Trends Immunol. 29:479.
6. McLaren, J.E. and D.P. Ramji (20 09) Cytokine Grwoth Factor Rev. 20:125.
7. Gray, P.W. and D.V. Goeddel (1982) Nature 298:859.
8. Bach, E.E. et al. (1997) Annu. Rev. Immunol. 15:563.
9. Marsters, S.A. et al. (1995) Proc. Natl. Acad. Sci. 92:5401.
10. Krause, C.D. et al. (2000) J. Biol. Chem. 275:22995.
11. Sugaya, M. et al. (1999) J. Invest. Dermatol. 113:350.
12. Howie, S.E.M. et al. (1996) J. Invest. Dermatol. 106:1218.
13. Battistini, L. et al. (1997) J. Immunol. 159:3723.
14. Gupta, A.A. et al. (1996) J. Immunol. 157:2123.
15. Neumann, H. et al. (1997) J. Exp. Med. 186:2023.
16. Hoiden, I. & G. Moller (1996) Scand. J. Immunol. 44:501.
17. Puddu, P. et al. (1997) J. Immunol. 159:3490.
18. Yoshimoto, T. et al. (1997) Proc. Natl. Acad. Sci. USA 94:3948.
19. Yeaman, G.R. et al. (1998) J. Immunol. 160:5145.
20. Asea, A. et al. (1996) Clin. Exp. Immunol. 105:376.
21. Briscoe, D.M. et al. (1997) J. Immunol. 159:3247.
22. Dejuco, N. et al. (1995) Endocrinology136:4925.

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

