PRODUCT INFORMATION & MANUAL

Human IFN-γ Valukine™ ELISA

VAL104C

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

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version202006.1

TABLE OF CONTENTS

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/γδT cells (11), keratinocytes (12), peripheral blood γδT 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 monoclonal 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 monoclonal 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, serum and plasma.
- 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)	62.4	125.1	514	62	126	527
Standard Deviation	2.6	3.8	14.2	4.0	7.4	34.2
CV%	4.2	3.0	2.8	6.5	5.9	6.5

B. RECOVERY

The recovery of human IFN-γ spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range**
Cell culture media (n=4)	94	87 - 102%
Serum (n=4)	98	91 - 107%
Plasma (n=4)	89	80 - 97%

C. SENSITIVITY

The minimum detectable dose (MDD) of human IFN-γ is typically less than 2.91 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 HEK293-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) = 0.009 × Valukine Human IFN-γ value (pg/mL)

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human IFN-γ in various matrices and diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Serum (n=4)	Plasma (n=4)
1:2	Average % of Expected	101	102	104
	Range (%)	102 - 104	91 - 114	97 - 108
1:4	Average % of Expected	107	102	93
	Range (%)	106 - 109	92 - 119	90 - 97
1:8	Average % of Expected	107	94	90
	Range (%)	104 - 112	82 - 102	87 - 93
1:16	Average % of Expected	105	87	89
	Range (%)	105 - 116	86 - 88	86 - 91

F. SAMPLE VALUES

Cell Culture Supernates - Peripheral blood mononuclear cells from a single donor (PBMCs; seeded at 1 x 10^6 /mL) were cultured in RPMI 1640 supplemented with 10%

fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were left untreated or treated with 10 µg/mL of PHA for 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-γ.

Condition	(pg/mL)
Unstimulated	ND
Stimulated with PHA	78,480

ND=Non-Detectable

$CD4^+$ T cells were isolated from human PBMCs (from a single donor) using the MagCellect Human $CD4^+$ T cell Isolation Kit (R&D Systems® Catalog# MAGH102). $CD4^+$ T cells were then seeded at 5 x 10^5 /mL and cultured in ExCellerate Human T Cell Expansion Media, Xeno-Free (R&D Systems® Catalog# CCM030). T cells were left unstimulated in culture media, or cultured with 10 ng/mL GMP recombinant human (rh) IL-7 (R&D Systems® Catalog# 207-GMP) and 10 ng/mL GMP rhIL-15 (R&D Systems® Catalog# 247-GMP) and stimulated using immobilized Human CD3 epsilon Antibody (R&D Systems® Catalog# MAB100, coated at 1 ug/mL) with 5 ug/mL soluble Human CD28 Antibody (R&D Systems® Catalog# MAB342) or 25 µL Cloudz CD3/CD28 (Cloudz™ T Cell Activation Kit CD3/CD28, R&D Systems®) per mL of cultured media for 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-γ.

Condition	(pg/mL)
Untreated	ND
Stimulated with CD3/CD28 antibodies	49,500
Stimulated with Cloudz T Cell Activation Kit	186,800

ND=Non-Detectable

Sample value may vary from an individual donor.

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

G. SPECIFICITY

This assay recognizes natural and recombinant human IFN-γ.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human IFN-γ control were assayed for interference. No significant cross-reactivity or interference was observed

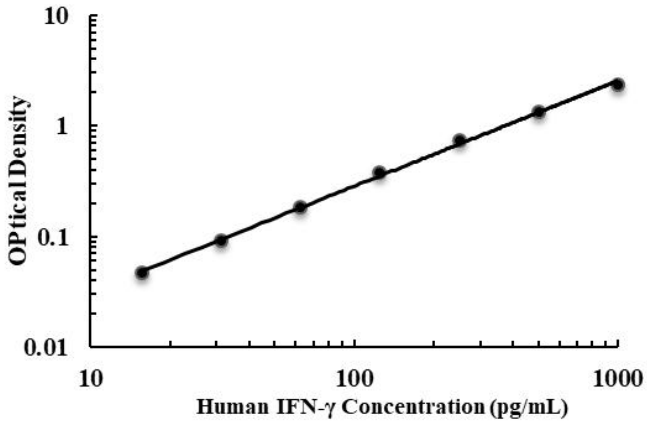
Recombinant human		**Recombinant mouse**
IFN-α1a	IFN-α16	IFN-γ
IFN-α1b	IFN-α17	IFN-γ R1
IFN-α2	IFN-α21	
IFN-α2a	IFNα2a + IFNα1b complex	**Other Recombinants**
IFN-α4a	IFN-β	bovine IFN-γ
IFN-α4b	IFN-γ R1	canine IFN-γ
IFN-α5	IFN-γ R2	cotton rat IFN-γ
IFN-α6	IL-28A	equine IFN-γ
IFN-α7	IL-28B	feline IFN-γ
IFN-α8	IL-29	porcine IFN-γ
IFN-α10		rat IFN-γ

Recombinant rhesus macaque IFN-γ interferes at concentrations > 5ng/mL and cross-reacts approximately 1.6% in this assay.

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	0.023 0.023	0.023	-
15.6	0.069 0.071	0.070	0.047
31.3	0.113 0.115	0.114	0.091
62.5	0.203 0.210	0.207	0.1835
125	0.393 0.399	0.396	0.373
250	0.749 0.760	0.755	0.7315
500	1.363 1.365	1.364	1.341
1000	2.360 2.372	2.366	2.343

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IFN-γ Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human IFN-γ	1 plate
Human IFN-γ Conjugate	a solution of monoclonal antibody against human IFN-γ conjugated to horseradish peroxidase	1 vial
Human IFN-γ Standard	recombinant human IFN-γ in a buffered protein base; lyophilized	1 vial
Calibrator Diluent (5×)	a 5× concentrated buffered protein base	1 vial
Assay Diluent	A buffered protein base with preservatives.	1 vial
Wash Buffer Concentrate (25×)	a 25× 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

<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="9">Opened/
Reconstituted
Reagents</td><td>Diluted Wash Solution</td><td rowspan="7">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Calibrator Diluent 1×</td></tr>
<tr><td>Assay Diluent</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at <-20°C. in a manual defrost freezer.
* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Microplate Wells</td><td>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.*</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 Calibrator 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.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20 °C. 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 Buffer Concentrate (25×) 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.

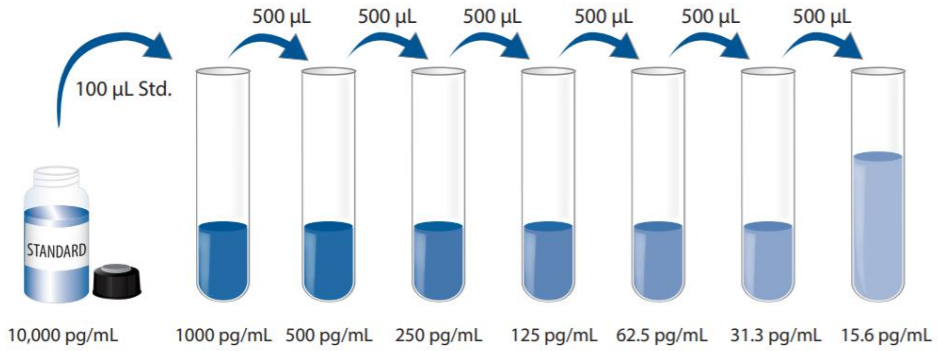
Calibrator Diluent 1× - Add 20 mL of Calibrator Diluent 5× into 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent 1×.

IFN-γ Standard - **Refer to the vial label for reconstitution volume for reconstitution***. This reconstitution produces a stock solution of 10,000 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 900 µL of Calibrate Diluent 1× into the 1000 pg/mL tube. Pipette 500µL into the remaining tubes. 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 Calibrate Diluent 1× serves as the zero standard (0pg/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 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 Assay Diluent to each well.
4. Add 100 µL of Standard, sample, or control per well. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** 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 200 µL of Human 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light**.
9. 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.
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

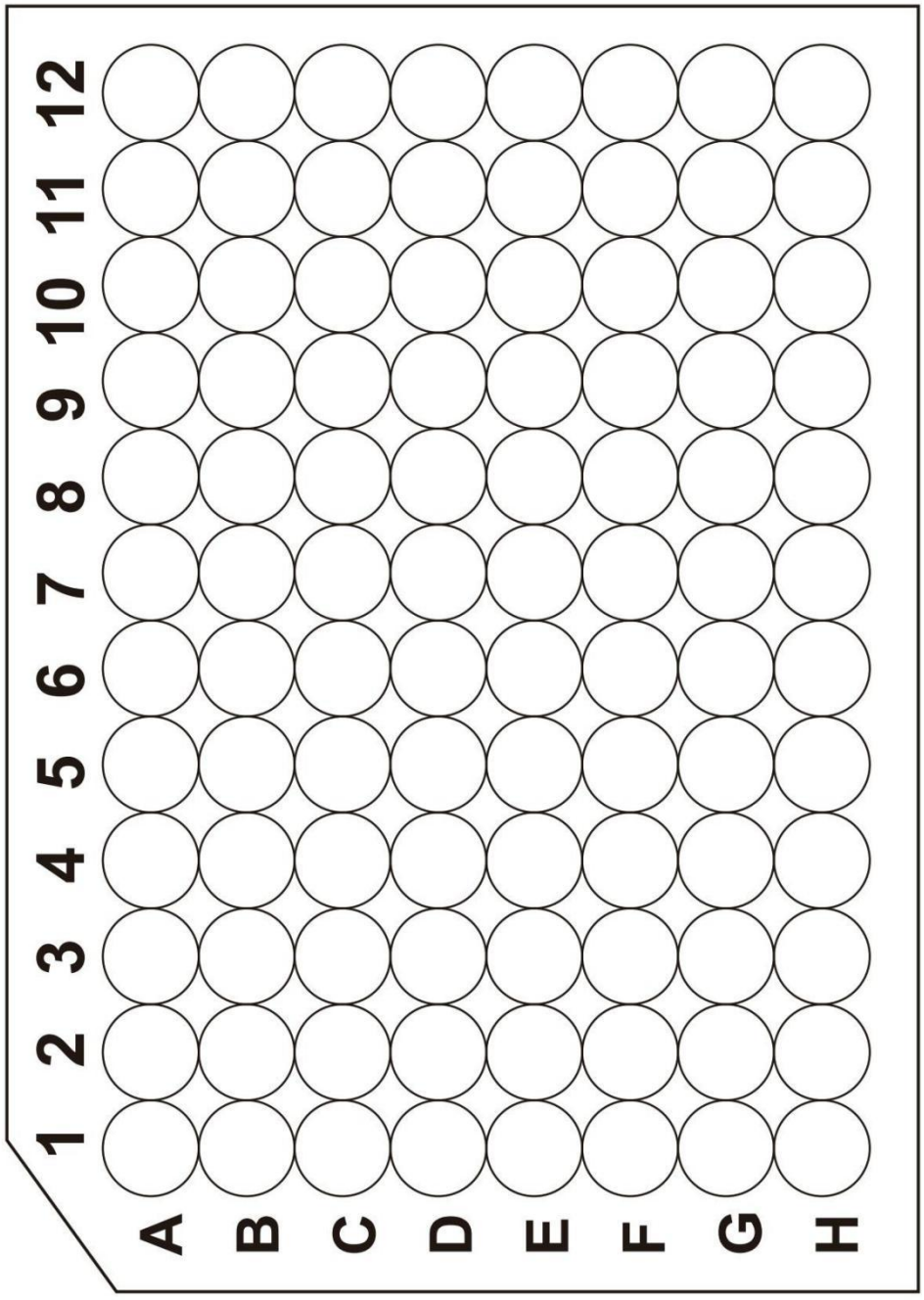
standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for 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. 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 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.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 IFN-γ Valukine™ ELISA 试剂盒

目录号：VAL104C

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

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

目录

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/γδ T细胞（11）、角化细胞（12）、外周血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）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单克隆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；加入辣根过氧化酶标记的抗人 IFN-γ单克隆抗体，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、人血清和血浆样本；
- 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。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	62.4	125.1	514	62	126	527
标准差	2.6	3.8	14.2	4.0	7.4	34.2
CV%	4.2	3.0	2.8	6.5	5.9	6.5

B. 回收率

在不同类型样本中掺入检测范围内不同水平的人IFN-γ，测定其回收率。

样本类型	平均回收率	范围
细胞培养上清 (n=4)	94	87 - 102%
血清 (n=4)	98	91 - 107%
血浆 (n=4)	89	80 - 97%

C. 灵敏度

人IFN-γ的最低可测值一般小于2.91 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Hek293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) = 0.009 × Valukine Human IFN-γ value (pg/mL)

E. 线性

在不同类型样本中掺入高浓度的人IFN-γ，然后用稀释剂将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养上清 (n=4)	血清 (n=4)	血浆 (n=4)
1:2	平均值/期待值（%）	101	102	104
	范围 (%)	102 - 104	91 - 114	97 - 108
1:4	平均值/期待值（%）	107	102	93
	范围 (%)	106 - 109	92 - 119	90 - 97
1:8	平均值/期待值（%）	107	94	90
	范围 (%)	104 - 112	82 - 102	87 - 93
1:16	平均值/期待值（%）	105	87	89
	范围 (%)	105 - 116	86 - 88	86 - 91

F. 样本预值

细胞上清样本 - 将一个捐赠者的外周血单核细胞（PBMCs；以1×10^6/mL接种）培养在添加10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100μg/mL硫酸链霉素的RPMI 1640中。细胞未经处理或用10μg/mL PHA处理5天。取细胞培养上清液等分，测定人IFN-γ水平。

条件	(pg/mL)
未刺激	ND
PHA刺激	78,480

ND=不可测

使用MagCellect人CD4⁺T细胞分离试剂盒（R&D Systems® Catalog#MAGH102）从人外周血单核细胞中分离出CD4⁺T细胞。CD4+T细胞以5×10⁵/mL细胞数接种于卓越无异源人T细胞扩展培养基中（R&D Systems®Catalog#CCM030）。T细胞在培养基中不受刺激，或和10 ng/mL GMP重组人（rh）IL-7（R&D Systems® Catalog# 207-GMP）和10 ng/mL GMP rh IL-15（R&D Systems® Catalog# 247-GMP）一起培养，并使用固定化人CD3-ε抗体（R&D Systems® Catalog# MAB100, 1 ug/mL包被）和5 ug/mL可溶性人CD28抗体（R&D Systems® Catalog# MAB342）或者25 µL/mL Cloudz CD3/CD28(Cloudz™ T Cell Activation Kit CD3/CD28, R&D Systems®)刺激5天。取细胞培养上清液等分，测定人IFN-γ水平。

条件	**(pg/mL)**
未刺激	ND
CD3/CD28抗体刺激	49,500
Cloudz T Cell Activation Kit 刺激	186,800

ND=不可测

样本值可能因捐赠者不同而存在差异。

血清/血浆样本 - 使用本试剂盒检测了4份人血清和4份人血浆样本中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

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×）配置成 50ng/mL 的浓度来检测与人IFN-γ的交叉反应。将50ng/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。

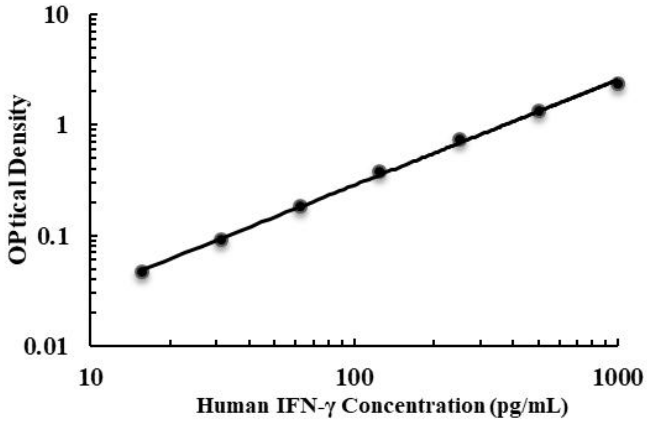
重组人蛋白		**重组小鼠蛋白**
IFN-α1a	IFN-α16	IFN-γ
IFN-α1b	IFN-α17	IFN-γ R1
IFN-α2	IFN-α21	
IFN-α2a	IFNα2a + IFNα1b complex	**其他重组蛋白**
IFN-α4a	IFN-β	bovine IFN-γ
IFN-α4b	IFN-γ R1	canine IFN-γ
IFN-α5	IFN-γ R2	cotton rat IFN-γ
IFN-α6	IL-28A	equine IFN-γ
IFN-α7	IL-28B	feline IFN-γ
IFN-α8	IL-29	porcine IFN-γ
IFN-α10		rat IFN-γ

重组猕猴IFN-γ干扰浓度大于5ng/mL，交叉反应率约为1.6%。

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	0.023 0.023	0.023	-
15.6	0.069 0.071	0.070	0.047
31.3	0.113 0.115	0.114	0.091
62.5	0.203 0.210	0.207	0.1835
125	0.393 0.399	0.396	0.373
250	0.749 0.760	0.755	0.7315
500	1.363 1.365	1.364	1.341
1000	2.360 2.372	2.366	2.343

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
人IFN-γ Microplate	包被单克隆抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
人IFN-γ Conjugate	酶标检测人IFN-γ单克隆抗体	1 瓶
人IFN-γ Standard	标准品（冻干粉）	1 瓶
Calibrator Diluent（5×）	浓缩稀释剂（5×）	1 瓶
Assay Diluent	检测液	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	终止液	1 瓶
Plate Covers	封板胶纸	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="8">已打开，稀释或重溶的试剂</td><td>洗涤缓冲液（1×）</td><td rowspan="6">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>检测液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td><td>分装，-20℃储存最多 30 天*；避免反复冻融。</td></tr>
<tr><td>标准品</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>
<tr><td>包被的微孔板条</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
- 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℃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。

血浆样本: 使用EDTA、肝素钠或枸橼酸钠作为抗凝剂收集血浆。然后1000 x g离心15分钟，需在30分钟内收集血浆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. 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℃。

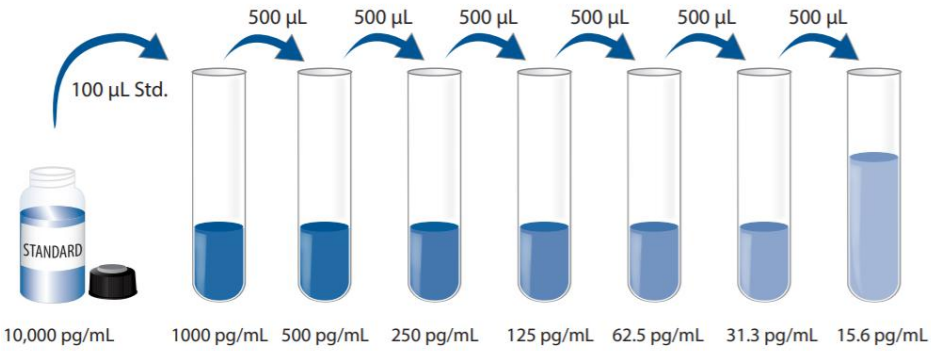
显色剂：按当次试验所需要用量将显色剂A和显色剂B等体积混合，避光；在使用前15分钟内准备，仅供当日使用；每孔需200μL。

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：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10,000pg/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。

使用聚丙烯试管作为标准品稀释管。在1000 pg/mL的稀释管中加入900μL稀释剂（1×），其余每个稀释管中加入500μL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（0pg/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. 在每个微孔内加入100μL检测液.
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100μL。**确保试剂添加过程不间断，并在15分钟内完成。**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200μL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200μL显色底物，室温孵育30分钟。**注意避光**；
9. 在每个微孔内加入50μL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30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

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