PRODUCT INFORMATION & MANUAL

Human IFN-β Valukine™ ELISA

VAL137

For the quantitative determination of natural and recombinant
Human IFN-β 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

Version202009.1

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I. BACKGROUND

Interferon beta (IFN-β), also known as fibroblast IFN, is a secreted, approximately 22 kDa member of the type I interferon family of molecules (1). Mature human IFN-β shares 47% and 46% amino acid sequence identity with the mouse and rat proteins, respectively. Fibroblasts are the major producers of IFN-β, but it can also be produced by dendritic cells, macrophages, and endothelial cells in response to pathogen exposure (2). It is transcriptionally regulated by TRAF3, IRF3, IRF7, and NFκB (3, 4). It has also been shown that the RIPK1 and RIPK3 kinases play a role in LPS-induced upregulation of IFN-β in mice (5). Following secretion, IFN-β signals through the heterodimeric IFN-α/β Receptor and activates the JAK/STAT signaling pathway (6-9). IFN-β appears to have a complex role in the regulation of inflammasomes. It has been shown to directly inhibit NLRP1 and NLRP3 inflammasomes in a STAT1-dependent manner and increase the susceptibility of mice to C. albicans infection (10). In contrast, L. monocytogenes has been shown to activate the NLRP3 inflammasome in an IFN-β-dependent manner (11). Viral infection of human mini-gut organoids induces IFN-β which leads to upregulation of Viperin and IFIT1 IFN-stimulated genes (12).

IFN-β-deficient mice show increased susceptibility to experimental autoimmune encephalomyelitis (EAE), a disease model of human multiple sclerosis (MS) (13). Furthermore, IFN-β has been shown to suppress the Th17 cell response in both MS and EAE and has commonly been used as a treatment for MS (14-18). Low levels of IFN-β have been associated with the hyporesponsive state of monocytes from sepsis patients, suggesting that IFN-β may have a role in restoring monocyte function and reversing immunosuppression (19). Type I IFNs (IFN-α and IFN-β) appear to have a role in the polarization of neutrophils in cancer. Inflammation, along with functional type I IFN signaling, was shown to alter neutrophil polarization towards anti-tumor phenotype (20).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human IFN-β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human IFN-β present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human IFN-β are pipetted into the wells. After washing away any unbound substances, streptavidin-HRP are pipetted into the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IFN-β 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 human 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 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)	16.3	53.2	192.4	16.6	53.0	188.9
Standard Deviation	0.6	1.0	4.6	1.0	1.1	7.6
CV%	3.7	1.9	2.4	6.0	2.1	4.0

B. RECOVERY

The recovery of human IFN-β spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 84.0-122.6% with an average of 98.1%.

The recovery of Human IFN-β spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 85.3-120.1% with an average of 97.1%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human IFN-β is typically less than 0.175 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 CHO cell-expressed recombinant human IFN-β produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human IFN-β and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	109.3	97.5-119.2
1:4	108.0	95.4-120.5
1:8	104.5	88.1-122.8
1:16	102.6	82.0-123.3

F. SAMPLE VALUES

Serum - Four human serum samples were evaluated for the presence of human IFN-β in this assay. All samples measured less than the lowest human IFN-β standard, 7.8 pg/mL.

G. SPECIFICITY

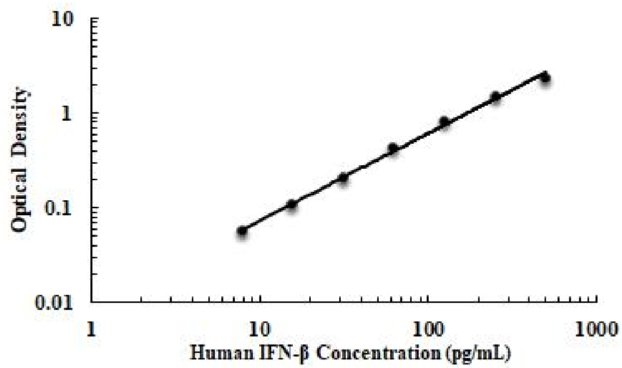
The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human	Recombinant Mouse
IFN-α / β R1	IFN-β
IFN-α / β R2/Fc Chimera	
IFN-ε	
IFN-δ 2	
IFN-γ	
IL-6	

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	0.040 0.041	0.041	-
7.8	0.095 0.098	0.097	0.056
15.6	0.148 0.149	0.149	0.108
31.3	0.245 0.248	0.247	0.206
62.5	0.460 0.469	0.465	0.424
125	0.832 0.899	0.866	0.825
250	1.539 1.544	1.542	1.501
500	2.334 2.385	2.360	2.319

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IFN-β Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human IFN-β.	1 plate
Human IFN-β Standard	Recombinant human IFN-β in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human IFN-β Detection Antibody	Biotinylated IFN-β antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (1×)	Concentrated buffered diluent used to dilute standard and samples.	2 vials
Detection Antibody Diluent (4×)	Concentrated buffered diluent used to dilute Detection Antibody.	1 vial
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	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 Sealers	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="11">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="5">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody Diluent (4×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.</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

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- 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 ≤ -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.

B. SAMPLE PREPARATION

Human serum samples require a 4-fold dilution. A suggested 4-fold dilution is 50 μL of sample + 150 μL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

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

Wash Buffer - 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.

Detection Antibody Diluent (1×) - Use deionized or distilled water to prepare Detection Antibody Diluent (1×).

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

Detection Antibody- Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Detection Antibody Diluent (1×) to the working concentration of 250 ng/mL. Prepare at least 15 minutes prior to use.

Streptavidin-HRP B (1×) **-** Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

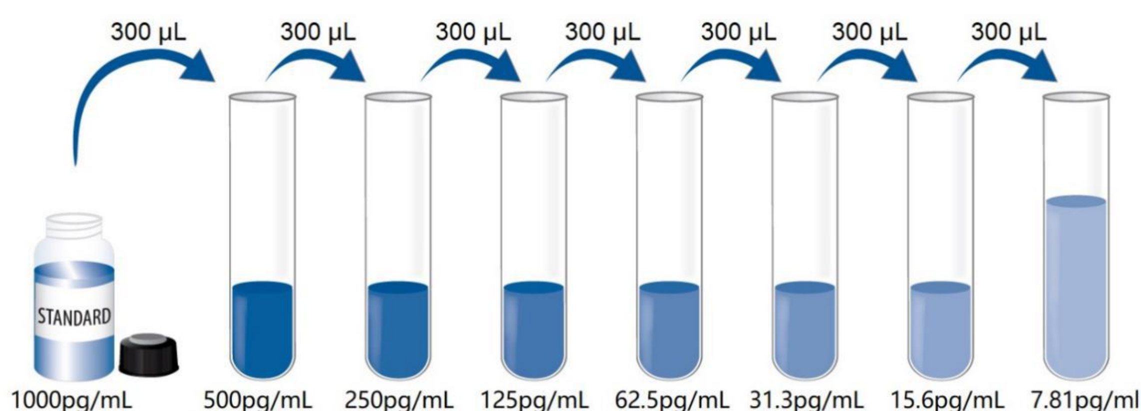
Substrate Solution - Color Reagent A and B should be mixed together in equal

volumes within 15 minutes of use. Protect from light. 100 μL of the resultant mixture is required per well.

Human IFN-β Standard - Refer to the vial label for the reconstitution volume*. 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.

Pipette 300 μL of the appropriate Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The Calibrator Diluent (1×) 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, 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 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 100 μL of the Detection Antibody diluted in Detection Antibody Diluent, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
10. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, 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, control, and 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 IFN-β 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.

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

产品信息及操作手册

人 IFN-β Valukine^{TM} ELISA 试剂盒

目录号：VAL137

适用于定量检测天然和重组人 IFN-β 的浓度

科研专用，不可用于临床诊断

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P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

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I. 背景

β干扰素(IFN-β)，又称成纤维细胞IFN，是一种分泌型，约22 kDa的I型干扰素家族成员(1)。成熟人IFN-β与小鼠和大鼠蛋白的氨基酸序列同源性分别为47%和46%。IFN-β主要由成纤维细胞产生，但它也可以由树突状细胞、巨噬细胞和内皮细胞产生，以应对病原体暴露 (2)。它由TRAF3、IRF3、IRF7和NFκB转录调控(3, 4)。也有研究表明，RIPK1和RIPK3激酶在LPS诱导的小鼠IFN-β上调中发挥作用(5)。分泌后，IFN-β通过异二聚体IFN-α/β受体发出信号并激活JAK/STAT信号通路(6-9)。IFN-β在炎症小体的调节中起着复杂的作用。它被证明以STAT1依赖的方式直接抑制NLRP1和NLRP3炎症小体，并增加小鼠对白色念珠菌感染的易感性(10)。相反，单核球增多性李斯特菌已经证明以IFN-β依赖的方式激活NLRP3炎症小体(11)。病毒感染人类微肠道器官可诱导IFN-β，从而导致Viperin和IFIT1 IFN刺激基因的上调(12)。

IFN-β缺陷型小鼠对实验性自身免疫性脑脊髓炎(EAE)的易感性增加，EAE是人类多发性硬化症(MS)的疾病模型(13)。此外，IFN-β在MS和EAE中都能抑制Th17细胞的反应，并且通常被用来治疗MS (14-18)。低水平的IFN-β与脓毒症患者单核细胞的低反应状态有关，提示IFN-β可能在恢复单核细胞功能和逆转免疫抑制中发挥作用(19)。I型干扰素(IFN-α和IFN-β)似乎在肿瘤中性粒细胞极化中起作用。炎症和功能性I型IFN信号已被证明改变中性粒细胞向抗肿瘤表型的极化(20)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IFN-β抗体包被于微孔板上，样品和标准品中的人IFN-β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人IFN-β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，应将样本用标准品稀释剂（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。

	板内精确度			**板间精确度**		
样本	1	2	3	1	2	3
平均值（pg/mL)	16.3	53.2	192.4	16.6	53.0	188.9
标准差	0.6	1.0	4.6	1.0	1.1	7.6
CV%	3.7	1.9	2.4	6.0	2.1	4.0

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人IFN-β，测定其回收率。回收率范围在84.0-122.6%，平均回收率在98.1%。

在人血清样本中掺入检测范围内不同水平的人IFN-β，测定其回收率。回收率范围在85.3-120.1%，平均回收率在97.1%。

C. 灵敏度

人IFN-β的最低可测剂量（MDD）一般小于0.175pg/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试剂盒经由R&D Systems生产的CHO细胞表达的高纯度重组人IFN-β蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人IFN-β，然后用标准品稀释剂（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	109.3	97.5-119.2
1:4	108.0	95.4-120.5
1:8	104.5	88.1-122.8
1:16	102.6	82.0-123.3

F. 样本预值

血清样本 - 使用本试剂盒检测了4份人血清样本中IFN-β的水平。4份样本的检测值均低于最低标准品，7.8pg/mL。

G. 特异性

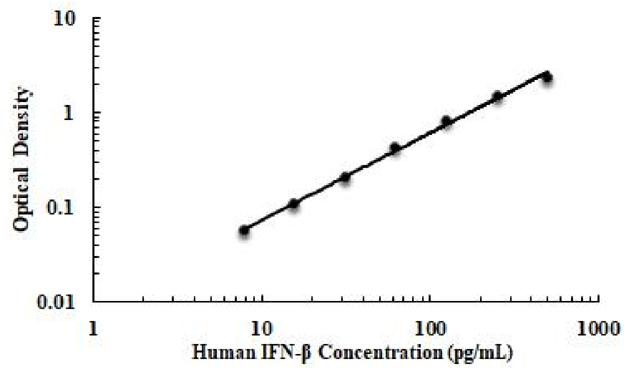
将以下因子配置成50ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

Recombinant human	Other recombinants
IFN-α/β R1	IFN-β
IFN-α/β R2/Fc Chimera	
IFN-ε	
IFN-δ2	
IFN-γ	
IL-6	

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。



pg/mL	OD	Average	Corrected
0	0.040 0.041	0.041	-
7.8	0.095 0.098	0.097	0.056
15.6	0.148 0.149	0.149	0.108
31.3	0.245 0.248	0.247	0.206
62.5	0.460 0.469	0.465	0.424
125	0.832 0.899	0.866	0.825
250	1.539 1.544	1.542	1.501
500	2.334 2.385	2.360	2.319

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IFN-β Microplate	包被小鼠抗人 IFN-β抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human IFN-β Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human IFN-β Detection antibody	生物素化的 IFN-β检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）	1 瓶
Calibrator Diluent (1×)	浓缩的标准品稀释剂（1×）	2 瓶
Detection Antibody Diluent (4×)	浓缩的检测抗体稀释液（4×）	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 Sealers	封板膜	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="12">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP B</td><td rowspan="5">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释剂（1×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×标准品稀释剂</td></tr>
<tr><td>检测抗体稀释液（4×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</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

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- Color Reagent B可能引起皮肤、眼睛和呼吸道刺激，避免吸入。
- 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。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。样本可能需要用标准品稀释剂（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1000xg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

人血清样本需要用标准品稀释剂（1×）4倍稀释后进行检测，例如：50μL样本+150μL标准品稀释剂（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℃。

检测抗体稀释液（1×）：使用蒸馏水或去离子水稀释配置成检测抗体稀释液（1×）。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配置成试剂稀释液（1×）。

检测抗体：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。再用检测抗体稀释液（1×）稀释至工作浓度250ng/mL，至少在使用前15分钟准备。

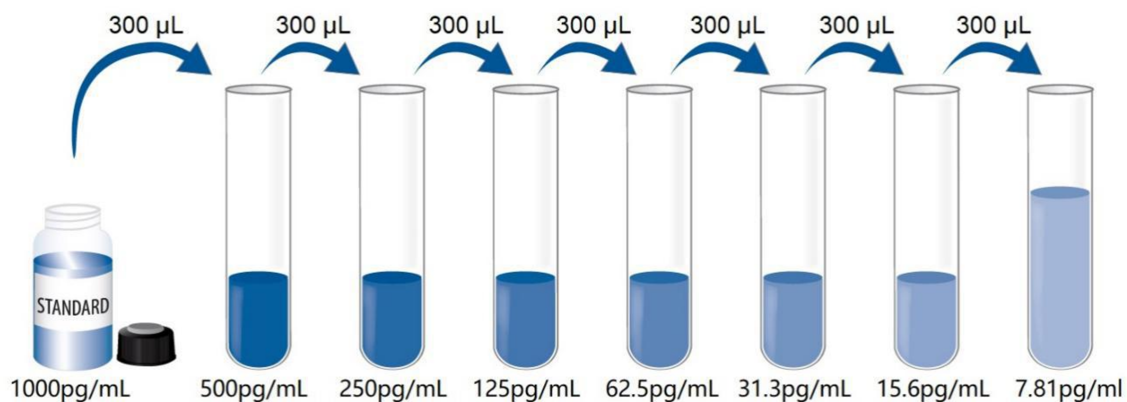
链霉亲和素- HRP B：用试剂稀释液（1×）将链霉亲和素- HRP B（40×）稀释至工作浓度链霉亲和素- HRP B（1×）。

显色剂：按试验所需用量（100μL/孔）将显色液A和显色液B等体积混合，避光保存，现用现配，须在15分钟内使用。

IFN-β 标准品：冻干标准品的重溶体积请参考瓶身标签，得到浓度为1000pg/mL标准品母液。轻轻震摇至少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300μL标准品稀释剂（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。500pg/mL管作标准曲线最高点，标准品稀释剂（1×）可用作标准品零点（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；
- 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100μL。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400μL，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100μL配置好的检测抗体。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100μL稀释好的链霉亲和素- HRP B工作液。用封板膜封住反应孔，室温孵育20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100μL显色剂，室温孵育30分钟，**注意避光**；
10. 在每个微孔内加入50μL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；
12. **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。通过样本的OD值，可从标准曲线上得到样本中人IFN-β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