PRODUCT INFORMATION & MANUAL

Human IFN-α2 Valukine™ ELISA

VAL169

For the quantitative determination of natural and recombinant human IFN-α2 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

Version202305.1

TABLE OF CONTENTS

I. BACKGROUND

There are 3 major classes of interferons (IFNs): Type I, Type II and Type III. Interferon alpha (IFN-α), along with IFN-β, IFN-δ, IFN-ε, IFN-κ, IFN-ω and IFN-τ are all Type I IFNs (1). The sole type II IFN is IFN-γ. Type III IFNs include IFN-λ1, IFN-λ2, IFN-λ3 and IFN-λ4 (2). As a part of the innate immune response, Type I IFNs are rapidly induced in response to viral nucleic acids such as double stranded DNA or RNA (dsDNA, dsRNA) and single stranded RNA (ssRNA), viral glycoproteins, microbial cytosine-phosphate-guanosine (CpG) DNA, DNA damage, and chromosomal instability (3,4).

IFN-α subtypes are well described (5-9). There are 16 human IFN-α subtypes with 80% amino acid identity (10). The number of IFN-α subtypes varies by species with 6 equine subtypes, 17 porcine subtypes, 14 bovine subtypes, and 9 canine subtypes known currently. Human IFN-α subtypes include: IFN-α1a, IFN-α1b, IFN-α2a, IFN-α2b, IFN-α2c, IFN-α4a, IFN-α4b, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α14, IFN-α16, IFN-α17, and IFN-α21. Although there is one known heterodimeric IFN-α receptor (IFN-αR, described below), each IFN-α subtype has been correlated with differing biological activities (7). Variability in biological responses can be attributed to differences in binding affinity and duration, receptor density, feedback responses and intracellular characteristics (11). IFN-α responses have been described as robust, especially in the context of viral infection responsiveness by all cells or tunable in a cell type specific manner.

IFN-α signaling is well characterized (2, 5,11,12,13). IFN-α is a ligand for IFNαR, which includes two subunits IFNαR1 and IFNαR2. IFN-α ligand binding to the ubiquitously expressed IFNαR1 triggers a conformational change which allows for the heterodimerization of IFNαR1 and IFNαR2 (10). Heterodimerization results in the cross phosphorylation of the Janus-activated Kinase 1 (JAK1) on IFNαR2 and tyrosine kinase 2 (TYK2) on IFNαR1 respectively, as well as the intracellular domain of IFNαR1 and IFNα2 (9). The transcription factors Signal transducer and activator of transcription (STAT) 1 and 2 are subsequently recruited to IFNαR via their Src homology 2 (SH2) domain and phosphorylated. In the canonical IFN-α signaling pathway, phosphoSTAT1/STAT2 heterodimers associate with Interferon Regulatory Factor 9 (IRF9) to form Interferon Stimulated Gene Factor 3 (ISGF3), which translocates into the nucleus to bind to gamma-activated sequences (GASs) or interferon- stimulated response elements (ISREs). This stimulates the transcription of interferon-stimulated genes (ISGs). STAT 2 homodimers and monomers can also associate with IRF-9 to form ISGF3-like complex, which also binds to ISRE to stimulate the transcription of ISGs (9).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human IFN-α2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human IFN-α2 present is bound by the immobilized antibody. A biotinylated detection antibody specific for human IFN-α2 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 (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human IFN-α2 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 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 (1×) or Calibrator Diluent-S (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

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)	5.9	22.8	92.1	6.0	24.1	96.7
Standard Deviation	0.2	0.9	3.6	0.2	2.4	7.6
CV%	3.9	3.8	3.9	4.0	9.9	7.8

B. RECOVERY

The recovery of human IFN-α2 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 84.7 to 119.3% with an average of 101.2%.

The recovery of human IFN-α2 spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 84.4 to 122.5% with an average of 101.6%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human IFN-α2 is typically less than 0.18 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 HEK293 expressed recombinant human IFN-α2 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-α2 and diluted with Calibrator Diluent (1×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples*) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	100.3	92.7-112.1
1:4	102.3	88.0-105.8
1:8	102.9	89.0-111.6
1:16	102.1	87.7-110.4

F. SAMPLE VALUES

Serum - Six human serum samples were evaluated for the presence of human IFN-α2 in this assay. All samples measured less than the lowest standard, 3.1 pg/mL.

G. SPECIFICITY

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

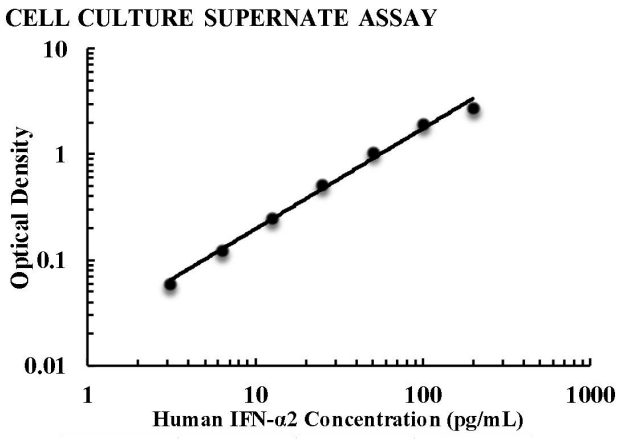
Recombinant human:		**Recombinant mouse:**
IFN-α1A	IFN-α21	IFN-α2
IFN-α1B	IFN-α/β R1	
IFN-α4B	IFN-α/β R2/Fc Chimera	
IFN-α5	IFN-β	

Recombinant Human Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
IFN-α 2A	156.3	78	50.2
IFN-α 4A	312.5	44	13.9
IFN-α 6	78.1	32	40.5
IFN-α 7	2500	26	1.0
IFN-α 8	312.5	30	9.6
IFN-α 10	312.5	52	16.8
IFN-α 14	1250	36	2.9
IFN-α 16	312.5	21	6.8
IFN-α 17	312.5	34	10.7

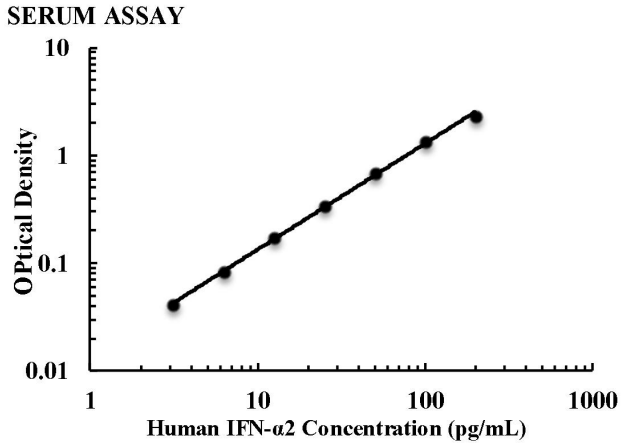
IV. EXPERIMENT

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	0.020 0.022	0.021	-
3.1	0.079 0.081	0.080	0.059
6.3	0.142 0.144	0.143	0.122
12.5	0.269 0.273	0.271	0.250
25	0.531 0.533	0.532	0.511
50	1.049 1.054	1.052	1.031
100	1.942 1.947	1.945	1.924
200	2.764 2.777	2.771	2.750



pg/mL	OD	Average	Corrected
0	0.019 0.020	0.020	-
3.1	0.059 0.061	0.060	0.041
6.3	0.102 0.102	0.102	0.083
12.5	0.185 0.196	0.191	0.171
25	0.359 0.363	0.361	0.342
50	0.697 0.718	0.708	0.688
100	1.341 1.367	1.354	1.335
200	2.332 2.349	2.341	2.321

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IFN-α2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human IFN-α2.	1 plate
Human IFN-α2 Standard	Recombinant human IFN-α2 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human IFN-α2 Detection Antibody	Biotinylated human IFN-α2 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent Concentrate (4×)	A 4× concentrated buffered diluent used to dilute standard and cell culture supernate samples.	1 vial
Calibrator Diluent-S (1×)	A buffered diluent used to dilute standard and serum samples.	2 vials
Detection Antibody Diluent Concentrate (10×)	A 10× concentrated buffered diluent used to dilute Detection Antibody.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered protein base used to dilute HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution.	1 vial
Stop Solution	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="9">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP A</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.
Standards may be stored for up to 1 month at -20 °C.*</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 Concentrate (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 Concentrate (4×)</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>Calibrator Diluent-S (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Detection Antibody Diluent Concentrate (10×)</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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

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 ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples 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. Samples require dilution with Calibrator Diluent-S (1×).

B. SAMPLE PREPARATION

Serum samples recommend a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent-S (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: Bring all reagents to room temperature before use.

Wash Buffer (1×) - 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 (1×).

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

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 - Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the detection antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Detection Antibody Diluent (1×). Prepare at least 15 minutes prior to use.

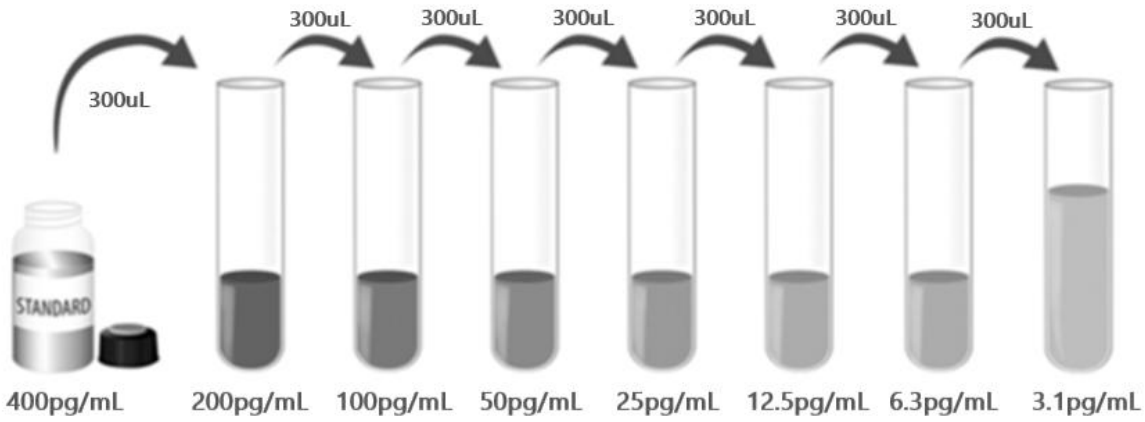
Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Human IFN-α2 Standard - Centrifuge briefly before opening. Refer to the vial label

for the reconstitution volume*. This reconstitution produces a stock solution of 400 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×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 pg/mL standard serves as the high standard. The Calibrator Diluent (1×) (*for cell culture supernate samples*) or Calibrator Diluent-S (1×) (*for serum samples*) 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 µL of standard, or prepared sample per well. And add 50 µL of the Detection Antibody (1×) diluted in Detection Antibody Diluent, to each well. Cover with the adhesive strip provided. **Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker set at 500 ± 50rpm.** 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 working dilution of Streptavidin-HRP A to each well. Cover the plate and **incubate for 30 minutes at room temperature on a horizontal orbital microplate shaker set at 500±50rpm. Protect from light**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature on a horizontal orbital microplate shaker set at 500 ± 50rpm. Protect from light**.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. 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.

10. 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 human IFN-α2 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.

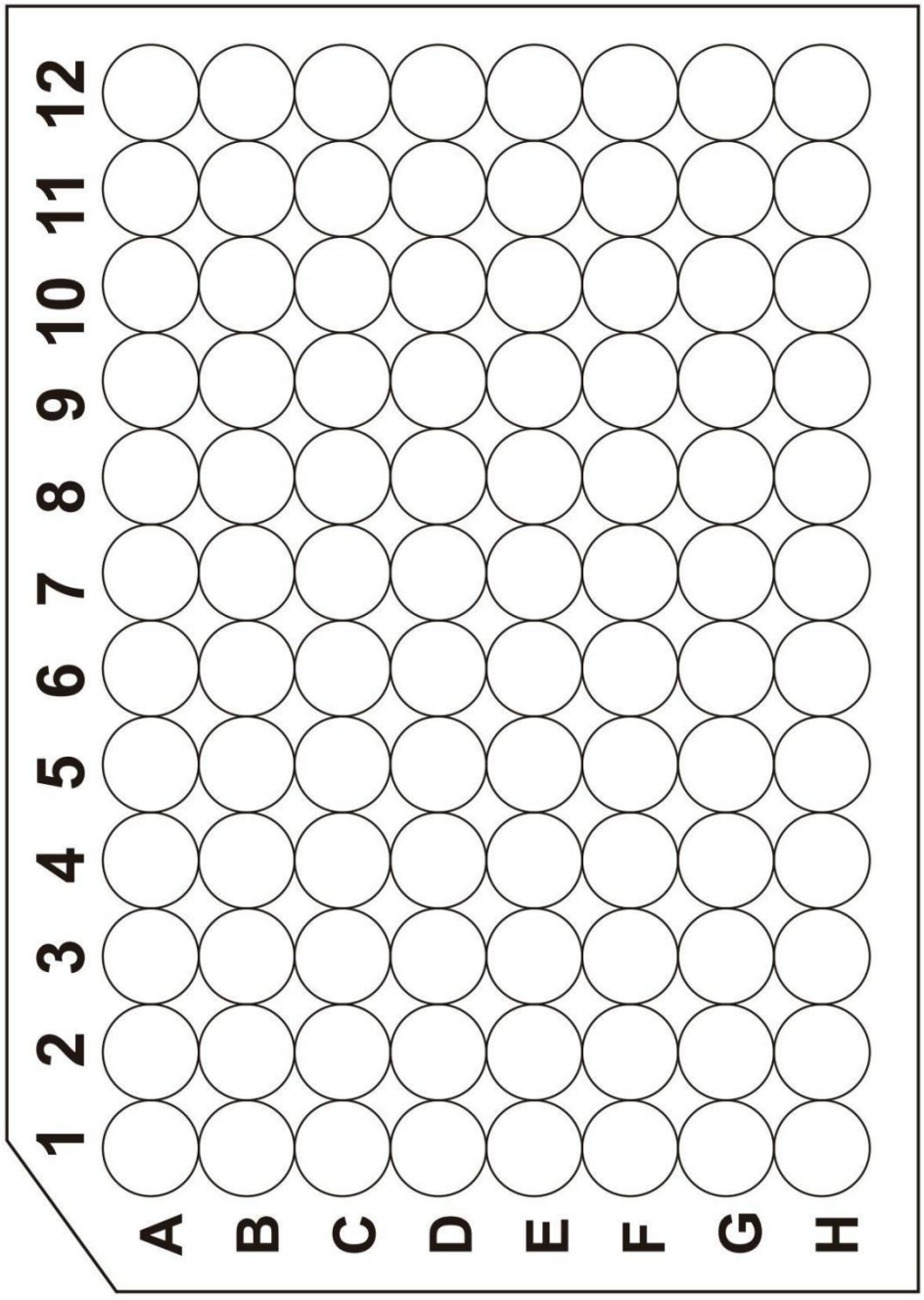
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Murira, A. & A. Lamarre (2016) Front. Immunol. 7:1
2. Lazear, H. M. et al. (2019) Immunity. 50:909
3. Tomasello, E. et al. (2014) Front. Immunol. 5:1
4. Fuertes, M.B. et al. (2013) Trends Immunol. 34:67
5. Ivashkiv, L. B. et al. (2014) Nat. Rev. Immunol. 14:36
6. Li, S. et al. (2018) Cell. Phys. And Biochem. 51:2377
7. Gibbert, K. et al. (2010) Br. J of Pharm. 168:1048
8. Moll, H. P. et al. (2011) Cytokine. 53:52
9. Provance, O. K. & J. Lewis-Wambi (2019) Breast Cancer Res. 21:59
10. Paul, F. et al. (2015) Gene. 567:132
11. Schreiber, G. (2017) JBC. 292:7285
12. Heise, R. et al. (2015) PLOS One. 11:1
13. Blaszczyk, K. et al. (2016) Cytokine & Growth Factors Rev. 29:71

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 IFN-α2 Valukine™ ELISA 试剂盒

目录号：VAL169

适用于定量检测天然和重组人 IFN-α2 的浓度

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

干扰素(IFN)有三大类:I型、II型和III型。干扰素α (IFN-α)、IFN-β、IFN-δ、IFN-ε、IFN-κ、IFN-ω和IFN-τ都是I型IFN (1)。唯一的II型IFN是IFN-γ。III型IFN包括IFN-λ1、IFN-λ2、IFN-λ3和IFN-λ4 (2)。作为先天免疫反应的一部分，I型IFN在响应病毒核酸如双链DNA或RNA (dsDNA、dsRNA)和单链RNA (ssRNA)、病毒糖蛋白、微生物胞嘧啶-磷酸-鸟苷(CpG) DNA、DNA损伤和染色体不稳定性时被迅速诱导(3, 4)。

IFN-α亚型有很好的描述(5-9)。有16种人类IFN-α亚型具有80%的氨基酸同源性(10)。IFN-α亚型的数量因物种而异，目前已知的有6种马亚型、17种猪亚型、14种牛亚型和9种犬亚型。人类IFN-α亚型包括：IFN-α1a、IFN-α1b、IFN-α2a、IFN-α2b、IFN-α2c、IFN-α4a、IFN-α4b、IFN-α5、IFN-α6、IFN-α7、IFN-α8、IFN-α10、IFN-α14、IFN-α16、IFN-α17和IFN-α21。虽然有一种已知的异二聚体IFN-α受体(IFN-αR，如下所述)，但每种IFN-α亚型都与不同的生物活性相关(7)。生物反应的可变性可归因于结合亲和力和持续时间、受体密度、反馈反应和细胞内特征的差异(11)。IFN-α反应被认为是稳健的，特别是在所有细胞对病毒感染的反应或以细胞类型特定的方式进行调节的情况下。

IFN-α信号传导具有良好的特征(2, 5, 11, 12, 13)。IFN-α是IFN-αR的配体，包括IFNαR1和IFNαR2两个亚基。IFN-α配体与普遍表达的IFN-αR1结合触发构象变化，从而允许IFNαR1和IFNαR2的异二聚化(10)。异源二聚化导致IFNαR2上的Janus活化激酶1(JAK1)和IFNαR1上的酪氨酸激酶2 (TYK2)以及IFNαR1和IFNα2的胞内结构域的交叉磷酸化(9)。转录因子信号换能器和转录激活因子(STAT) 1和2随后通过其Src同源性2 (SH2)结构域被募集到IFNαR并磷酸化。在典型的IFN-α信号通路中，phosphoSTAT1/STAT2异源二聚体与干扰素调节因子9 (IRF9)结合形成干扰素刺激基因因子3 (ISGF3)，该因子易位到细胞核中结合γ活化序列(GASs)或干扰素刺激反应元件(ISREs)。这会刺激干扰素刺激基因(ISGs)的转录。STAT 2同型二聚体和单体也可以与IRF-9结合形成ISGF3样复合体，该复合体也与ISRE结合以刺激ISGs的转录(9)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IFN-α2抗体包被于微孔板上，样品和标准品中的人IFN-α2会与固定在板上的抗体结合，接着加入生物素化的抗人IFN-α2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液（1×）或标准品稀释液-S（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)	5.9	22.8	92.1	6.0	24.1	96.7
标准差	0.2	0.9	3.6	0.2	2.4	7.6
CV%	3.9	3.8	3.9	4.0	9.9	7.8

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-α2，测定其回收率。回收率范围在84.7-119.3%，平均回收率在101.2%。

在血清样本中掺入检测范围内不同水平的人IFN-α2，测定其回收率。回收率范围在84.4-122.5%，平均回收率在101.6%。

C. 灵敏度

人IFN-α2的最低可测剂量（MDD）一般小于0.18 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试剂盒经由R&D Systems生产的高纯度HEK293表达的重组人IFN-α2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-α2，然后用标准品稀释液（1×）（*用于细胞上*

清样本）或标准品稀释液-S（1×）（*用于人血清样本*）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	100.3	92.7-112.1
1:4	102.3	88.0-105.8
1:8	102.9	89.0-111.6
1:16	102.1	87.7-110.4

F. 样本预值

血清样本 - 使用本试剂盒检测了6份人血清样本中IFN-α2的水平。6份样本的检测值均低于最低标准品，3.1 pg/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或干扰。

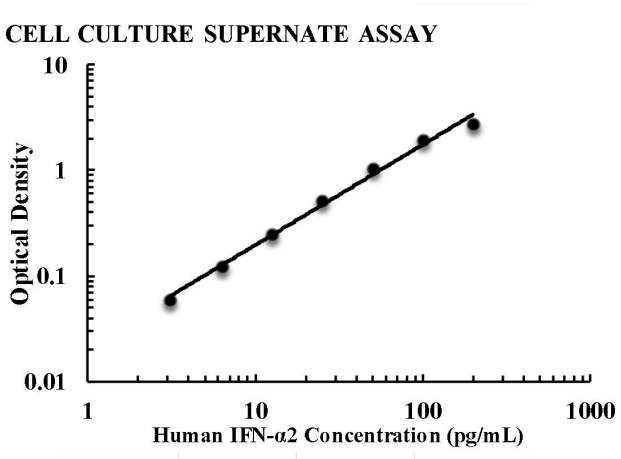
Recombinant human:		**Recombinant mouse:**
IFN-α1A	IFN-α21	IFN-α2
IFN-α1B	IFN-α/β R1	
IFN-α4B	IFN-α/β R2/Fc Chimera	
IFN-α5	IFN-β	

Recombinant Human Factor	Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
IFN-α 2A	156.3	78	50.2
IFN-α 4A	312.5	44	13.9
IFN-α 6	78.1	32	40.5
IFN-α 7	2500	26	1.0
IFN-α 8	312.5	30	9.6
IFN-α 10	312.5	52	16.8
IFN-α 14	1250	36	2.9
IFN-α 16	312.5	21	6.8
IFN-α 17	312.5	34	10.7

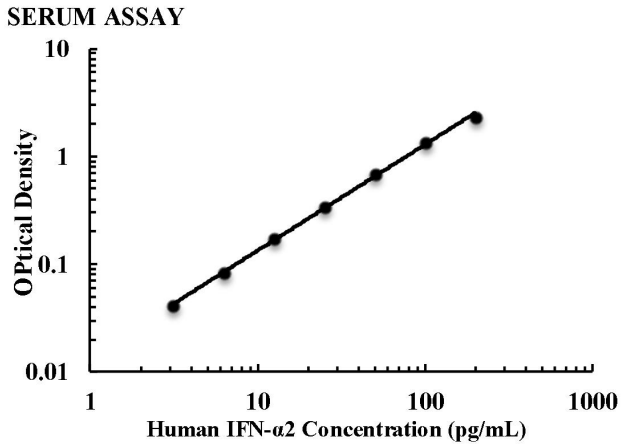
IV. 实验

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	0.020 0.022	0.021	-
3.1	0.079 0.081	0.080	0.059
6.3	0.142 0.144	0.143	0.122
12.5	0.269 0.273	0.271	0.250
25	0.531 0.533	0.532	0.511
50	1.049 1.054	1.052	1.031
100	1.942 1.947	1.945	1.924
200	2.764 2.777	2.771	2.750



pg/mL	OD	Average	Corrected
0	0.019 0.020	0.020	-
3.1	0.059 0.061	0.060	0.041
6.3	0.102 0.102	0.102	0.083
12.5	0.185 0.196	0.191	0.171
25	0.359 0.363	0.361	0.342
50	0.697 0.718	0.708	0.688
100	1.341 1.367	1.354	1.335
200	2.332 2.349	2.341	2.321

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IFN-α2 Microplate	包被小鼠抗人 IFN-α2 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human IFN-α2 Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human IFN-α2 Detection Antibody	生物素化的人 IFN-α2 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent Concentrate (4×)	浓缩的标准品稀释液（4×）用于稀释标准品和细胞上清样本	1 瓶
Calibrator Diluent-S (1×)	标准品稀释液（1×）用于稀释标准品和血清样本	2 瓶
Detection Antibody Diluent Concentrate (10×)	浓缩的检测抗体稀释液（10×）用于稀释检测抗体	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释 HRP	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate 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="10">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*
标准品-20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</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>标准品稀释液（4×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×标准品稀释液</td></tr>
<tr><td>标准品稀释液-S（1×）</td><td>2-8℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液</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
- 振荡器（速度可调至 500±50 rpm）

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。样本可能需要用标准品稀释液-S（1×）稀释。

B. 样本准备工作

血清样本建议用标准品稀释液-S（1×）4倍稀释后进行检测，例如：50 µL样本+150 µL标准品稀释液-S（1×）。最佳稀释度应由最终用户确定。

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浓缩洗涤液用去离子水或蒸馏水稀释配制成500mL工作浓度的洗涤液（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×）。

检测抗体稀释液（1×）：使用去离子水或蒸馏水稀释配制成检测抗体稀释液（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×）。

检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备 检测抗体（100×）。轻轻震摇至少15分钟，使其充分溶解。如有需要分装保存。用检测抗体稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

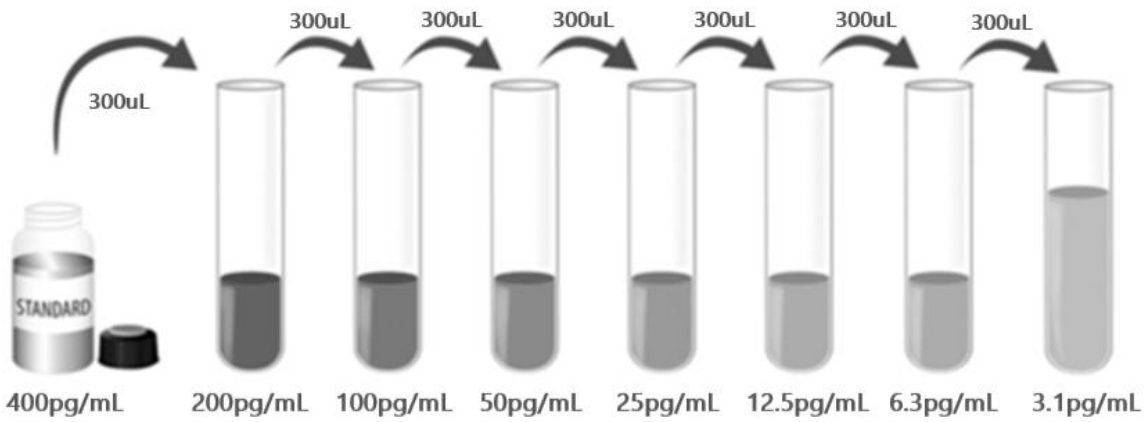
链霉亲和素- HRP A：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

人IFN-α2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400 pg/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300 µL标准品稀释液（1×）（*用于细胞上清样本*）或标准品稀释液-S（1×）（*用于人血清样本*）。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。200 pg/mL管作标准曲线最高点，标准品稀释液（1×）（*用于细胞上清样本*）或标准品稀释液-S（1×）（*用于人血清样本*）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，TMB底物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。然后每个微孔内加入50 µL配置好的检测抗体（1×），用封板膜封住反应孔，**室温500±50rpm水平振荡孵育3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500±50rpm水平振荡孵育30分钟。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 TMB底物溶液，**室温500±50rpm水平振荡孵育30分钟。注意避光；**
8. 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；
9. 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；
10. **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-α2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

1. Murira, A. & A. Lamarre (2016) Front. Immunol. 7:1
2. Lazear, H. M. et al. (2019) Immunity. 50:909
3. Tomasello, E. et al. (2014) Front. Immunol. 5:1
4. Fuertes, M.B. et al. (2013) Trends Immunol. 34:67
5. Ivashkiv, L. B. et al. (2014) Nat. Rev. Immunol. 14:36
6. Li, S. et al. (2018) Cell. Phys. And Biochem. 51:2377
7. Gibbert, K. et al. (2010) Br. J of Pharm. 168:1048
8. Moll, H. P. et al. (2011) Cytokine. 53:52
9. Provance, O. K. & J. Lewis-Wambi (2019) Breast Cancer Res. 21:59
10. Paul, F. et al. (2015) Gene. 567:132
11. Schreiber, G. (2017) JBC. 292:7285
12. Heise, R. et al. (2015) PLOS One. 11:1
13. Blaszczyk, K. et al. (2016) Cytokine & Growth Factors Rev. 29:71

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