PRODUCT INFORMATION & MANUAL

Human CXCL10/IP-10 Valukine™ ELISA

Catalog Number: VAL208

For the quantitative determination of natural and recombinant human Interferon gamma inducible Protein 10 (IP-10) concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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

Version 202410.1

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I. BACKGROUND

IP-10 (interferon-gamma inducible protein 10 kDa), also known as CXCL10, was originally identified as an IFN-γ-inducible gene. It is induced in a variety of cells in response to IFN-γ and LPS. In contrast to other CXC chemokines, IP-10 has no chemotactic activity for neutrophils. It is a pleiotropic molecule that appears to target activated T cells and monocytes (1-3). IP-10 inhibits bone marrow colony formation and angiogenesis (4, 5). It can also stimulate NK and T cell migration, regulate T cell maturation and modulate adhesion molecule expression (for a review, see reference 6).

IP-10 cDNA encodes a 98 amino acid (aa) precursor protein with a 21 aa signal peptide that is cleaved to generate a 77 aa mature protein (1). The aa sequence of IP-10 indicates that it is a member of a subfamily of CXC chemokines lacking the ELR domain.

CXCR3 is a receptor for both IP-10 and MIG (7, 8). It is highly expressed in IL-2-activated T lymphocytes and can also be expressed on eosinophils (9), yet is undetectable in resting T lymphocytes, B lymphocytes, monocytes or granulocytes. CXCR3 can also be expressed on $CD34^+$ hematopoietic progenitors from human cord blood stimulated with GM-CSF, but not on freshly isolated $CD34^+$ progenitor cells (10). CXCR3 promotes Ca^{2+} mobilization and chemotaxis specifically in response to IP-10 and MIG, and not to other CXC or CC chemokines (7).

IP-10 mRNA is expressed by activated T lymphocytes, neutrophils, splenocytes, keratinocytes, osteoblasts, astrocytes, endothelial cells, and smooth muscle cells (11). It is also expressed in inflammatory skin diseases and cutaneous T cell lymphomas.

IP-10 expression has been associated with HIV infection. It can contribute to the accumulation of activated T cells in the cerebrospinal fluid compartment in HIV-1 infected individuals (12). The retroviral transactivator, HIV-1 Tat, is a potent inducer of IP-10 expression in astrocytes (13). Tat can induce expression levels of IP-10 sufficient to promote chemotaxis of peripheral blood lymphocytes. This Tat-mediated IP-10 mRNA induction can be suppressed by a mitogenactivated protein kinase (MAPK) inhibitor, thus indicating that MAPKs play a major role in Tatmediated chemokine induction in astrocytes.

IP-10 expression has also been shown to be significantly elevated in astrocytes within the brains of Alzheimer's Disease patients (14). Astrocytes expressing IP-10 are commonly associated with senile plaques. The receptor for IP-10, CXCR3, can be detected constitutively on neurons and neuronal processes in various cortical and subcortical regions of the brain.

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II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human IP-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human IP-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human IP-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human IP-10 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, human serum, human plasma, human saliva and human urine.
- 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 RD5K/RD6Q 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 forty separate assays to assess inter-assay precision.

CELL CULTURE SUPERNATE/HUMAN SALIVA/HUMAN URINE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	70.3	174	342	80.7	194	362
Standard Deviation	2.07	5.15	10.74	7.91	13.48	24.27
CV%	2.9	3.0	3.1	9.8	6.9	6.7

HUMAN SERUM/PLASMA ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	67.4	168	334	70.7	182	366
Standard Deviation	3.12	4.99	11.15	6.23	11.12	18.93
CV%	4.6	3.0	3.3	8.8	6.1	5.2

B. RECOVERY

The recovery of human IP-10 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell Culture Media (n=4)	95	88-101
Human Serum (n=5)	99	88-112
Human EDTA plasma (n=5)	99	90-109
Human Heparin plasma (n=5)	99	87-113

C. SENSITIVITY

Fifty-seven assays were evaluated and the minimum detectable dose (MDD) of human IP-10 ranged from 0.41-4.46 pg/mL. The mean MDD was 1.67 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified *E-coli*-expressed recombinant human IP-10 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 IP-10 and diluted with Calibrator Diluent RD5K/RD6Q to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture samples (n=9)	Human Serum (n=5)	Human EDTA plasma (n=9)	Human Heparin plasma (n=9)	Human Saliva (n=5)	Human Urine (n=4)
1:2	Average % of Expected	102	102	104	106	110	103
	Range (%)	95-106	92-107	95-113	101-112	106-118	101-106
1:4	Average % of Expected	102	106	104	108	112	106
	Range (%)	96-106	93-117	98-111	101-113	108-120	102-110
1:8	Average % of Expected	103	106	98	106	112	109
	Range (%)	97-107	99-112	94-111	97-112	108-127	101-117
1:16	Average % of Expected	102	106	102	105	117	110
	Range (%)	92-112	99-115	83-108	98-112	117-117	98-121

F. SAMPLE VALUES

Human Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human IP-10 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human Serum (n=60)	89	38-361
Human EDTA plasma (n=35)	96	47-382
Human Heparin plasma (n=35)	110	52-450
Human Saliva (n=5)	729	292-1340

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human Urine (n=18)	17.2	67	ND-49.7

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 mM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human IP-10.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	29,774	21,900
Stimulated	18,456	11,091

THP-1 human acute monocyte leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated with 1.0 µg/mL recombinant human IFN-γ for 8 hours and then 1.0 µg/mL LPS was added. Cells were incubated for an additional 18 hours. An aliquot of the cell culture supernate was removed, diluted 600-fold and assayed, and measured 164,640 pg/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human IP-10.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5K/RD6Q and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human IP-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

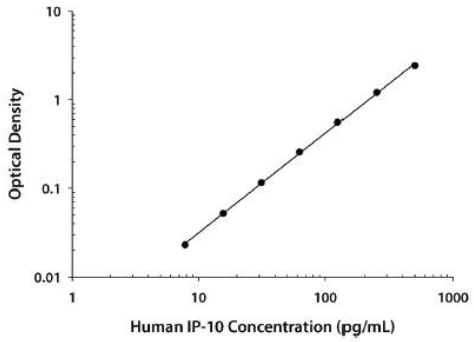
Recombinant human：	**Recombinant mouse:**	**Recombinant porcine:**
BLC/BCA-1	BLC/BCA-1	IL-8
ENA-78	CRG-2 (IP-10)	
GCP-2	GCP-2	
GROα	KC	
GROβ	MIG	
GROγ	SDF-1α	
IFN-γ		
IL-8		
IL-8, endothelial cell-derived		
I-TAC		
MIG		
NAP-2		
SDF-1α		
SDF-1β		

IV. EXPERIMENT

EXAMPLE STANDARD

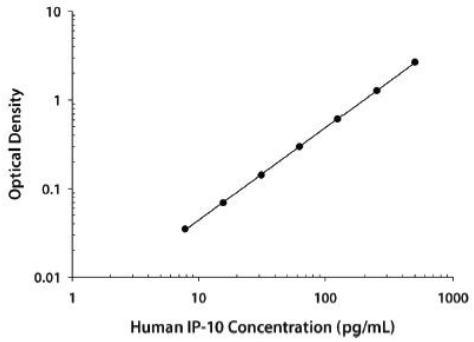
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.027	0.027	—
7.8	0.052 0.048	0.050	0.023
15.6	0.082 0.076	0.079	0.052
31.3	0.145 0.141	0.143	0.116
62.5	0.288 0.281	0.284	0.257
125	0.575 0.588	0.582	0.555
250	1.251 1.215	1.233	1.206
500	2.455 2.460	2.458	2.431

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.027	0.027	—
7.8	0.064 0.061	0.062	0.035
15.6	0.101 0.090	0.096	0.069
31.3	0.174 0.166	0.170	0.143
62.5	0.338 0.314	0.326	0.299
125	0.652 0.629	0.640	0.613
250	1.314 1.288	1.301	1.274
500	2.622 2.764	2.693	2.666

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IP-10 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IP-10	1 plate
Human IP-10 Conjugate	Solution of antibody against human IP-10 conjugated to horseradish peroxidase	1 vial
Human IP-10 Standard	Recombinant human IP-10 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1-56	A buffered protein base	1 vial
Calibrator Diluent RD5K	A buffered protein base used to dilute standard and samples (*For cell culture supernate/human saliva/human urine samples*)	1 vial
Calibrator Diluent RD6Q	A buffered protein base used to dilute standard and samples (*For human serum/human plasma samples*)	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
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="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Assay Diluent RD1-56</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD5K</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent RD6Q</td><td>May be stored for up to 1 month at 2-8 °C.*</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.
- 100 mL and 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 RD5K.

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 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6Q.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6Q.

Note: *Citrate plasma has not been validated for use in this assay.*

Grossly hemolyzed samples are not suitable for use in this assay.

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5K.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD5K.

B. SAMPLE PREPARATION

Most cell culture supernate samples recommend at least a 30-fold dilution. A suggested 30-fold dilution is 10 µL of sample + 290 µL of Calibrator Diluent RD5K. Optimal dilutions should be determined by the end user.

All human saliva samples recommend at least a 10-fold dilution. A suggested 10-fold dilution is 25 µL of sample + 225 µL of Calibrator Diluent RD5K. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

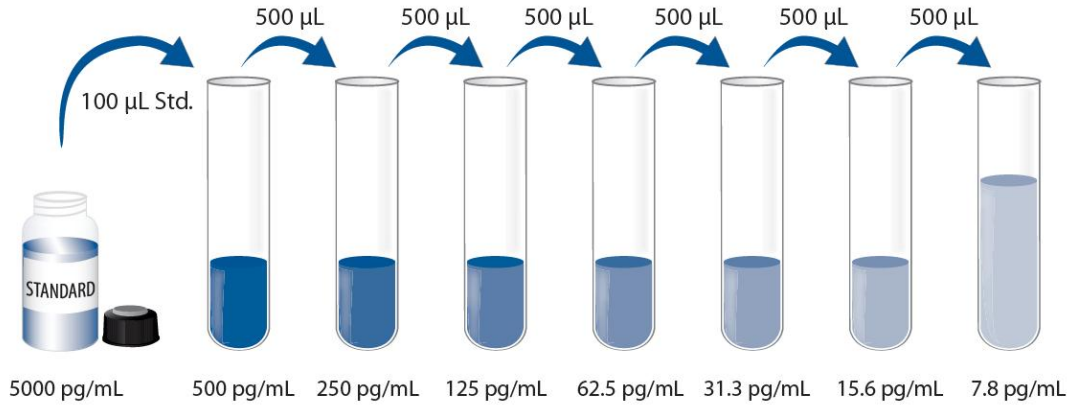
Note: Bring all reagents to room temperature before use.

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×).

Human IP-10 Standard - Refer to the vial label for the reconstitution volume* Reconstitute the Human IP-10 Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 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 Calibrator Diluent RD5K (*for cell culture supernate/human saliva/human urine samples*) or Calibrator Diluent RD6Q (*for human serum/plasma samples*) into the 500 pg/mL tube. Pipette 500 µL of the appropriate calibrator diluent into the remaining tubes. 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 appropriate calibrator diluent 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

Bring all other reagents and samples to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

Note: *High concentrations of human IP-10 are found in human saliva. Take precautionary measures to prevent contamination of kit reagents.*

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. **For Cell Culture Supernate/Human Saliva/ Human Urine samples:** Add 150 µL of Assay Diluent RD1-56 to each well.

   For Human Serum/Plasma samples: Add 75 µL of Assay Diluent RD1-56 to each well.
4. **For Cell Culture Supernate/Human Saliva/ Human Urine samples:** Add 100 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.**

   For Human Serum/Plasma samples: Add 75 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature.**
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (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 IP-10 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 TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. 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.

11. CALCULATION OF RESULTS

Average the duplicate readings for each standard 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 log/log 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 a log/log graph. The data may be linearized by plotting the log of the human IP-10 concentrations versus the log of the O.D. on a linear scale and the best fit line can be determined by regression analysis.

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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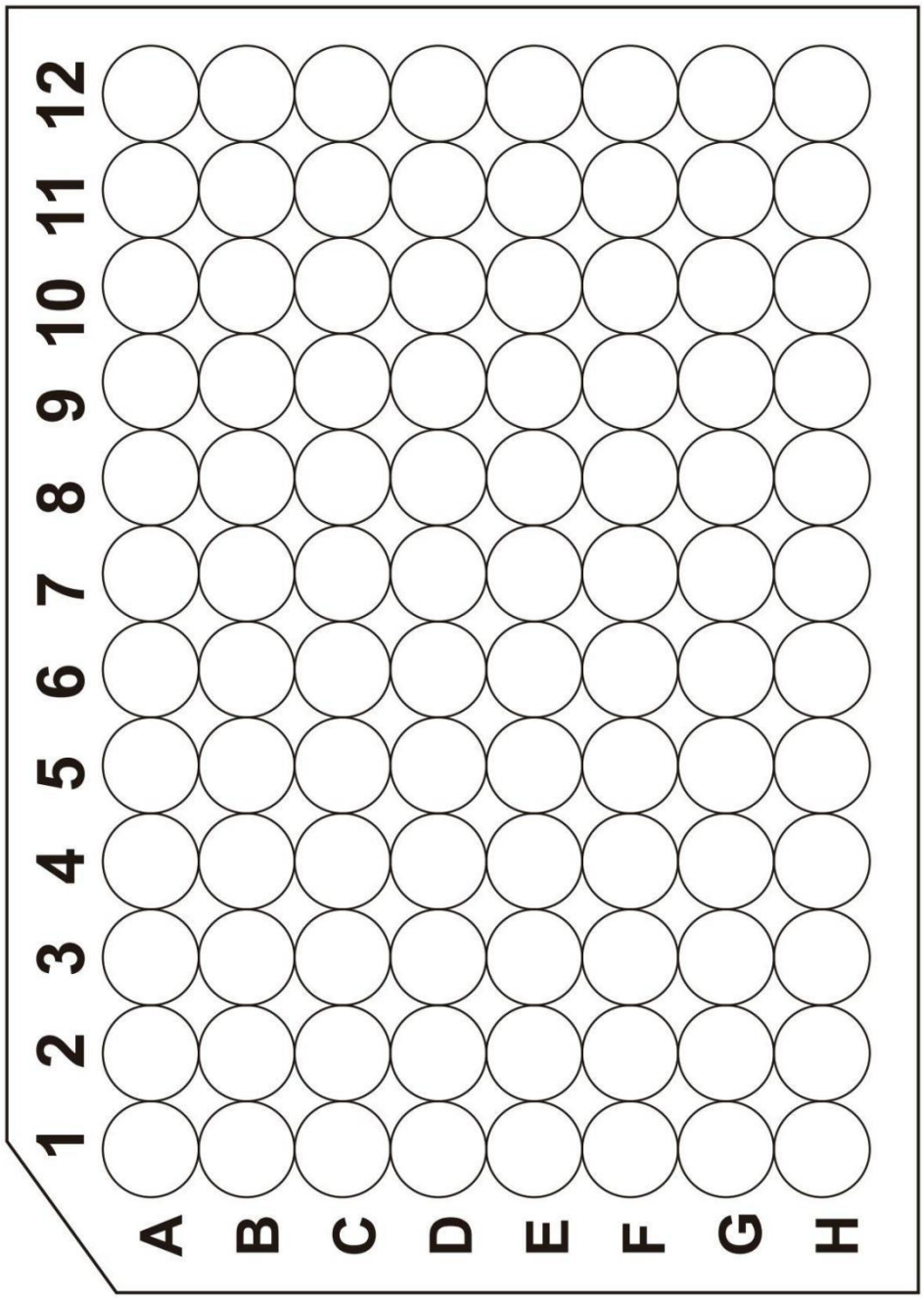
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 CXCL10/IP-10 Valukine™ ELISA 试剂盒

目录号：VAL208

适用于定量检测天然和重组人γ干扰素诱导蛋白 10 (IP-10) 的浓度

科研专用，不可用于临床诊断

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版本号 202410.1

目录

I. 背景

IP-10（干扰素-γ诱导蛋白 10 kDa）又称 CXCL10，被定义为 IFN-γ诱导基因。它能在多种细胞中对 IFN-γ和 LPS 产生诱导反应。与其他 CXC 趋化因子相比，IP-10 对中性粒细胞没有趋化活性。它是一种以活化的 T 细胞和单核细胞为靶标的多效应分子（1-3）。IP-10 可抑制骨髓集落形成和血管生成（4，5）。它还能刺激 NK 和 T 细胞迁移、调节 T 细胞成熟和调节粘附分子的表达（综述见参考文献 6）。

IP-10 cDNA 编码 98 个氨基酸 (aa) 的前体蛋白，其中有 21 aa 信号肽被裂解后生成 77 aa 的成熟蛋白（1）。IP-10 的 aa 序列表明，它是缺乏 ELR 结构域的 CXC 趋化因子亚家族成员。

CXCR3 是 IP-10 和 MIG 的受体（7，8）。它在 IL-2 激活的 T 淋巴细胞中高度表达，也可在嗜酸性粒细胞中表达（9），但在静止的 T 淋巴细胞、B 淋巴细胞、单核细胞或粒细胞中检测不到。CXCR3 也能在经 GM-CSF 刺激的人脐带血 $CD34^+$造血祖细胞上表达，但不能在新鲜分离的 $CD34^+$祖细胞上表达（10）。相对于其他 CXC 或 CC 趋化因子，IP-10 和 MIG 能够特异性地使 CXCR3 促进钙离子移动和趋化作用（7）。

活化的 T 淋巴细胞、中性粒细胞、脾脏细胞、角质形成细胞、成骨细胞、星形胶质细胞、内皮细胞和平滑肌细胞都表达 IP-10 mRNA（11）。在炎症性皮肤病和皮肤 T 细胞淋巴瘤中也有表达。

IP-10 的表达与 HIV 感染有关。它可导致 HIV-1 感染者脑脊液中活化 T 细胞的聚集（12）。逆转录病毒转录因子 HIV-1 Tat 是星形胶质细胞中 IP-10 表达的有效诱导因子（13）。Tat 可以诱导足够的 IP-10 的表达水平来促进外周血淋巴细胞的趋化。有丝分裂原激活蛋白激酶（mitogenactivated protein kinase，MAPK）抑制剂可抑制 Tat 介导的 IP-10 mRNA 诱导，从而表明 MAPKs 在 Tat 介导的星形胶质细胞趋化因子诱导过程中发挥了重要作用。

在阿尔茨海默病患者大脑中的星形胶质细胞中，IP-10 的表达也明显升高（14）。表达 IP-10 的星形胶质细胞通常与老年斑有关。在大脑皮层和皮层下各区域的神经元和神经过程中，可以连续检测到 IP-10，CXCR3 的受体。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IP-10抗体包被于微孔板上，样品和标准品中的人IP-10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IP-10检测抗体进行孵育。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液，人血清，人血浆，人唾液和人尿液；
- 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RD5K/RD6Q稀释并重复测定。
- 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、试剂盒的效期等。

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40次，以确定板间精确度。

细胞培养上清液/人类唾液/人类尿液检测

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	70.3	174	342	80.7	194	362
标准差	2.07	5.15	10.74	7.91	13.48	24.27
CV%	2.9	3.0	3.1	9.8	6.9	6.7

人血清/血浆检测

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	67.4	168	334	70.7	182	366
标准差	3.12	4.99	11.15	6.23	11.12	18.93
CV%	4.6	3.0	3.3	8.8	6.1	5.2

B. 回收率

不同类型样本中掺入检测范围内不同水平的人IP-10，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=4)	95	88-101
人血清 (n=5)	99	88-112
人EDTA血浆 (n=5)	99	90-109
人肝素血浆 (n=5)	99	87-113

C. 灵敏度

共评估了五十七次测试，人IP-10的最小可检测剂量（MDD）范围为0.41-4.46 pg/mL。平均MDD为1.67 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

该免疫测定法以R&D Systems生产的高纯度的大肠杆菌表达的重组人IP-10校正。

E. 线性

不同的样本中含有或掺入高浓度的人IP-10，然后用标准品稀释液RD5K/RD6Q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=9)	人血清 (n=5)	人EDTA血浆(n=9)	人肝素血浆 (n=9)	人唾液 (n=5)	人尿液 (n=4)
1:2	平均值/期待值（%）	102	102	104	106	110	103
	范围 (%)	95-106	92-107	95-113	101-112	106-118	101-106
1:4	平均值/期待值（%）	102	106	104	108	112	106
	范围 (%)	96-106	93-117	98-111	101-113	108-120	102-110
1:8	平均值/期待值（%）	103	106	98	106	112	109
	范围 (%)	97-107	99-112	94-111	97-112	108-127	101-117
1:16	平均值/期待值（%）	102	106	102	105	117	110
	范围 (%)	92-112	99-115	83-108	98-112	117-117	98-121

F. 样本预值

人血清/血浆/唾液/尿液 - 在本试验中评估了来自表面健康志愿者的样本中是否存在人 IP-10。本研究中使用的供体没有病史。

样本类型	平均值 (pg/mL)	范围 (pg/mL)
人血清 (n=60)	89	38-361
人 EDTA 血浆 (n=35)	96	47-382
人肝素血浆 (n=35)	110	52-450
人唾液 (n=5)	729	292-1340

样本类型	平均检测值 (pg/mL)	%可检测率	范围 (pg/mL)
人尿液 (n=18)	17.2	67	ND-49.7

ND=未检出

细胞培养上清：

人外周血细胞（1×10⁶个细胞/mL）在含有5%胎牛血清、50 mM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/毫升青霉素和100 µg/毫升链霉素硫酸盐的DMEM中培养。细胞在未刺激或用10 µg/mL PHA刺激后培养。取细胞培养上清液，测定人IP-10的水平。

条件	1 天 (pg/mL)	5 天 (pg/mL)
未刺激	29,774	21,900
刺激	18,456	11,091

THP-1人急性单核细胞白血病细胞在添加了10%胎牛血清、50 µM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL 硫酸链霉素的RPMI培养基中培养。细胞用1.0 µg/mL重组人IFN-γ刺激8小时后，再加入1.0 µg/mL LPS。细胞继续培养18小时。取细胞培养上清液，稀释600倍后进行检测，测得浓度为164,640 pg/mL。

G. 特异性

检测方法识别天然和重组人IP-10。

以下列出的因子在标准品稀释液RD5K/RD6Q中以50 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人IP-10 对照品中以50 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

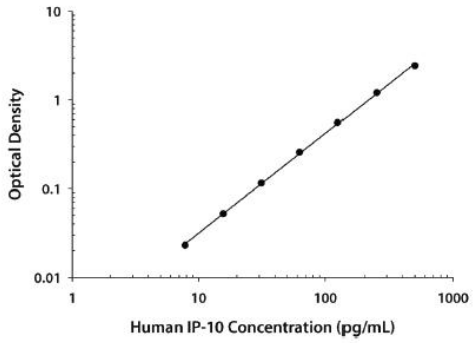
Recombinant human：	**Recombinant mouse:**	**Recombinant porcine:**
BLC/BCA-1	BLC/BCA-1	IL-8
ENA-78	CRG-2 (IP-10)	
GCP-2	GCP-2	
GROα	KC	
GROβ	MIG	
GROγ	SDF-1α	
IFN-γ		
IL-8		
IL-8, endothelial cell-derived		
I-TAC		
MIG		
NAP-2		
SDF-1α		
SDF-1β		

IV. 实验

标准曲线实例

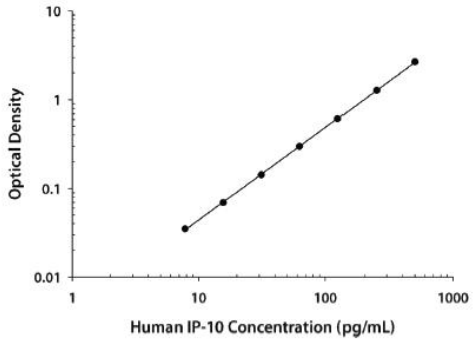
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE/SALIVA/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.027	0.027	—
7.8	0.052 0.048	0.050	0.023
15.6	0.082 0.076	0.079	0.052
31.3	0.145 0.141	0.143	0.116
62.5	0.288 0.281	0.284	0.257
125	0.575 0.588	0.582	0.555
250	1.251 1.215	1.233	1.206
500	2.455 2.460	2.458	2.431

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.027	0.027	—
7.8	0.064 0.061	0.062	0.035
15.6	0.101 0.090	0.096	0.069
31.3	0.174 0.166	0.170	0.143
62.5	0.338 0.314	0.326	0.299
125	0.652 0.629	0.640	0.613
250	1.314 1.288	1.301	1.274
500	2.622 2.764	2.693	2.666

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IP-10 Microplate	包被抗人IP-10抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human IP-10 Conjugate	酶标检测抗人IP-10抗体	1瓶
Human IP-10 Standard	重组人IP-10标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-56	检测液	1瓶
Calibrator Diluent RD5K	标准品稀释液，用于稀释标准品和样品（*用于细胞培养上清液/人唾液/人尿液样品*）	1瓶
Calibrator Diluent RD6Q	标准品稀释液，用于稀释标准品和样品（*用于人血清/人血浆样品*）	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

未开封试剂盒	2-8℃储存；请在试剂盒有效期内使用	
已打开，稀释或重溶的试剂	洗涤液（1×）	2-8℃储存，最多30天*
	终止液	
	酶标检测抗体	
	TMB底物溶液	
	标准品	分装并储存在 ≤ -20 °C 的冰箱中*。避免反复冻融循环。
	检测液RD1-56	2-8℃储存，最多30天*
	标准品稀释液RD5K	2-8℃储存，最多30天*
	标准品稀释液RD6Q	2-8℃储存，最多30天*
	包被的微孔板条	将未用的板条放回带有干燥剂的铝箔袋内，密封；2-8 ℃储存，最多30天*

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 100 mL和500 mL量筒

D. 注意事项

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

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液RD5K稀释。

血清 - 使用血清分离管（SST），让样本在室温下凝固 30 分钟，然后在 1000 × g 的离心力下离心 15 分钟。分离血清并立即进行检测，或分装并储存在 ≤ -20 °C。避免反复冻融循环。样品可能需要用标准品稀释液RD6Q进行稀释。

血浆 - 用EDTA或肝素作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在 ≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液RD6Q稀释。

注：*柠檬酸盐血浆在本检测中未经验证。*

严重溶血样品不适合用于该检测。

唾液 - 使用唾液采集器，如Salivette或等效设备采集唾液。立即检测或分装后存储于≤-20°C。避免反复冻融循环。样品可能需要用标准品稀释液RD5K进行稀释。

注：*唾液收集器不得具有任何蛋白质结合或过滤功能。*

尿液 - 无菌采集当日首次尿液（中段尿），直接排入无菌容器中。离心去除颗粒物质，立即检测或分装后储存于≤-20°C。避免反复冻融循环。样品可能需要用标准品稀释液RD5K进行稀释。

B. 样品准备

大多数细胞培养上清样品建议至少稀释 30 倍。建议的 30 倍稀释方法为：取 10 µL 样品 + 290 µL 标准品稀释液 RD5K 稀释。最佳稀释倍数应由用户自行确定。

所有人唾液样本建议至少进行 10 倍稀释。建议的 10 倍稀释方法为：25 µL 样品+225 µL 标准品稀释液 RD5K 稀释。最佳稀释倍数应由用户自行确定。

C. 检测前准备工作

注：*使用前请将所有试剂放置于室温。*

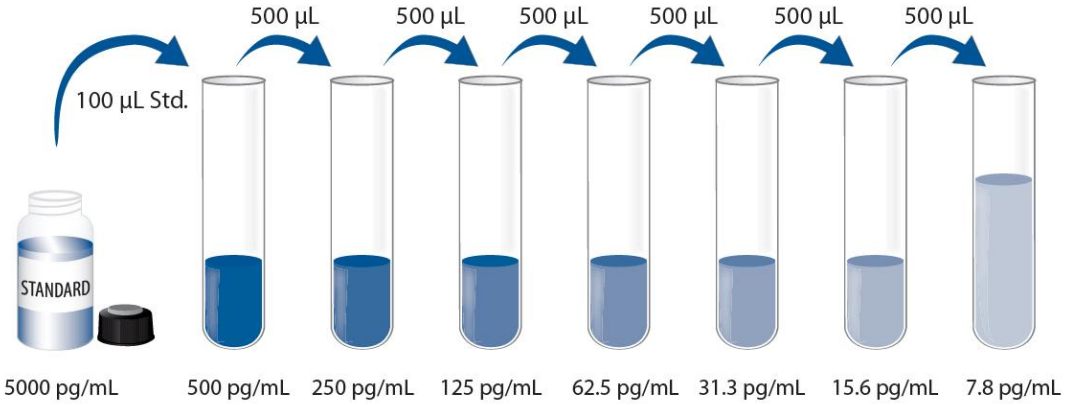
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

人IP-10标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重构人IP-10标准品，

得到浓度为5000 pg/mL标准品储备液。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。

使用聚丙烯管。将900 µL的标准品稀释液RD5K（*用于细胞培养上清液/人唾液/人尿液样本*）或标准品稀释液RD6Q（*用于人血清/血浆样本*）移入500 pg/mL的管子中。将适量的标准品稀释液500 µL移入剩余的管子中。将标准品储备液按下图稀释。在转移前充分混合每个管子。500 pg/mL的标准品作为最高标准品，标准品稀释液作为零标准（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄； 若孔内有绿色，则表明孔内液体未混匀请充分混合

VII.操作步骤

使用前，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

注：*人唾液中含有高浓度的人IP-10，请采取预防措施以防止试剂盒试剂受到污染。*

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. **细胞培养上清液/人唾液/人尿液样品：**向每个孔中加入150 µL检测液RD1-56。

   人血清/血浆样品：向每个孔中加入75 µL检测液RD1-56。
4. **细胞培养上清液/人唾液/人尿液样品：**每孔添加100 µL标准品和制备好的样品。用封板膜覆盖，**在室温下孵育2小时。**

   对于人血清/血浆样品：每孔添加75 µL标准品和制备好的样品。用封板膜覆盖，**在室温下孵育2小时。**
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；
6. 在每个微孔内加入200 µL人IP-10酶标检测抗体。用封板膜封住反应孔，**在室温下孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**；
9. 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；
10. 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。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
11. 计算结果：

    将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作log/log曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过log/log图上的点绘制最佳拟合曲线。数据可以通过绘制人IP-10浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。

    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