PRODUCT INFORMATION & MANUAL

Human Complement Factor D Valukine™ ELISA

Catalog Number: VAL226

For the quantitative determination of natural and recombinant human Complement Factor D 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

Version 202411.1

TABLE OF CONTENTS

I. BACKGROUND

The complement system comprises approximately 30 circulating plasma proteins as well as cell-surface receptors that function as part of the innate and adaptive immune system to eliminate pathogens. The system is organized into multiple proteolytic cascades where proteases exist as inactive zymogens and are activated by the action of an upstream active protease. Three pathways of complement activation exist: classical, lectin, and alternative. These pathways converge in the generation of the C3 convertase, which is responsible for the initiation of a series of events leading to the generation of bacterial opsonin (that facilitates the phagocytosis of opsonized pathogens), anaphylatoxins (that mediate inflammation), and the formation of the terminal membrane attack complex (that induces the lysis of pathogens or cells) (1).

Complement Factor D (factor D), also known as adipsin, is a serine protease that is indispensable for the initiation of complement activation by the alternative pathway. Upon activation through reversible substrate-induced conformational change into an active enzyme, factor D functions to cleave the C3b-bound factor B, resulting in the formation of C3bBb complex, which is the alternative pathway C3 convertase. Human complement factor D is synthesized as a 253 amino acid (aa) precursor that contains a signal peptide (aa 1-20), a fiveresidue activation-/pro-peptide (aa 21-25), and the mature chain (aa 26-253). Under physiological conditions, mature factor D lacking the activation peptide circulates as an inactive enzyme and requires interaction with its natural substrate, C3b-bound factor B, for activation of its catalytic activity (2). Mature human factor D shares 98%, 96%, 84%, and 66% aa sequence homology with the chimpanzee, rhesus monkey, porcine, and mouse protein, respectively. Factor D is expressed in multiple tissues, including monocyte/macrophages, muscle, sciatic nerve, endometrium, kidney, intestine, and at especially high levels in adipocytes (3). The level of factor D expression is reduced in various mouse models of obesity (4-6).

Serum complement factor D concentration is regulated through catabolism in the kidney where factor D is filtered by the glomerulus and reabsorbed by the proximal tubule (7). In patients with renal failure, circulating levels of factor D are elevated. Similarly in patients with Fanconi syndrome, a disorder in which the proximal tubular function of the kidney is impaired, urinary factor D concentrations are also highly elevated (8). Complement factor D deficiency is associated with low activity of the alternative complement pathway and low capacity to opsonize bacteria. In patients with mutations in the factor D gene resulting in complete factor D deficiency, recurrent bacterial infections were observed (9, 10).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Complement Factor D has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Complement Factor D present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Complement Factor D 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 Complement Factor D 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, human serum, human plasma 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 (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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (ng/mL)	0.860	3.12	8.13	0.831	3.04	7.73
Standard Deviation	0.047	0.183	0.552	0.074	0.211	0.506
CV%	5.5	5.9	6.8	8.9	6.9	6.5

B. RECOVERY

The recovery of human Complement Factor D spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	104	97-117
Human Urine (n=4)	100	87-115

C. SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human Complement Factor D ranged from 0.005-0.025 ng/mL. The mean MDD was 0.013 ng/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 NS0-expressed recombinant human Complement Factor D 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 Complement Factor D and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture supernates (n=4)	Human Serum* (n=4)	Human EDTA plasma* (n=4)	Human Heparin plasma* (n=4)	Human Urine (n=4)
1:2	Average % of Expected	100	103	103	96	103
	Range (%)	98-106	99-110	95-106	90-101	97-110
1:4	Average % of Expected	98	105	102	92	102
	Range (%)	89-113	94-112	89-115	91-94	96-104
1:8	Average % of Expected	92	100	101	92	101
	Range (%)	87-100	89-111	90-111	90-96	96-105
1:16	Average % of Expected	82	94	99	____	100
	Range (%)	____	85-103	93-105	____	99-100

*Samples were diluted prior to assay as directed in the Sample Preparation section.

F. SAMPLE VALUES

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

Sample Type	Mean of Detectable (ng/mL)	% Detectable (ng/mL)	Range (ng/mL)
Human Serum (n=35)	2339	100	1729-3238
Human EDTA plasma (n=35)	2297	100	1468-3657
Human Heparin plasma (n=35)	1667	100	906-2545
Human Urine (n=12)	1.83	75	ND-7.32

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood leukocytes (1 × 10^6 cells/mL) were cultured for 6 days in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human Complement Factor D, and measured 13.2 ng/mL.

Human peripheral blood mononuclear cells (1 × 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 ng/mL of recombinant human IL-2 for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of human Complement Factor D.

Condition	Day 1 (ng/mL)
Unstimulated	1.46
Stimulated	1.60

HT-29 human colon adenocarcinoma cells (0.25 × 10^5 cells/mL) were cultured in McCoy's 5a media supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 7 days. An aliquot of the

cell culture supernate was removed, assayed for human Complement Factor D, and measured 0.798 ng/mL.

U937 human histiocytic lymphoma cells (1×10^5 cells/mL) were cultured for 1 and 3 days in RPMI supplemented with 10% fetal bovine serum, and 2 mM L-glutamine. Aliquots of the cell culture supernates were removed and assayed for levels of human Complement Factor D.

Condition	Day 1 (ng/mL)	Day 3 (ng/mL)
Unstimulated	3.17	5.61

Dendritic cells (1×10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated with 1000 U/mL of recombinant human GM-CSF and 500 U/mL of recombinant human IL-4 for 7 days. An aliquot of the cell culture supernate was removed, assayed for human Complement Factor D, and measured 2.89 ng/mL.

G. SPECIFICITY

This assay recognizes natural and recombinant human Complement Factor D.

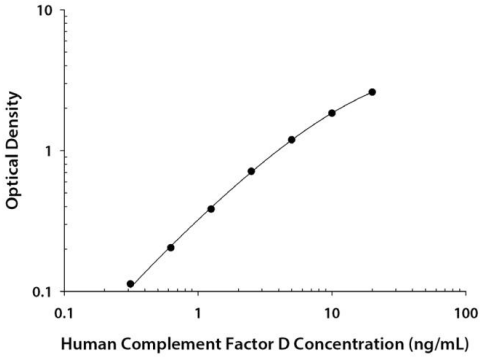
The factors listed below were prepared at 200 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human Complement Factor D control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	**Natural proteins:**
Coagulation Factor II/Thrombin	bovine Coagulation Factor Xa
Coagulation Factor VII	human Coagulation Factor II/Thrombin
Coagulation Factor X	
Coagulation Factor Xa	
Coagulation Factor XI	
Complement Component C1r	
Complement Component C1s	
Complement Component C2	
Complement Component C3	
Complement Factor B	
Complement Factor H-related 1/CFHR1	
Complement Factor H-related 5/CFHR5	

IV. EXPERIMENT

EXAMPLE STANDARD

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.015 0.017	0.016	—
0.313	0.125 0.132	0.129	0.113
0.625	0.217 0.223	0.220	0.204
1.25	0.394 0.406	0.400	0.384
2.50	0.720 0.736	0.728	0.712
5.00	1.200 1.210	1.205	1.189
10.0	1.840 1.880	1.860	1.844
20.0	2.590 2.650	2.620	2.604

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Complement Factor D Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Complement Factor D	1 plate
Human Complement Factor D Conjugate	Solution of antibody against human Complement Factor D conjugated to horseradish peroxidase	1 vial
Human Complement Factor D Standard	Recombinant human Complement Factor D in a buffered protein; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1W	A buffered protein base	1 vial
Calibrator Diluent Concentrate (4×)/ RD5-26	A 4× concentrated buffered protein base used to dilute standard and 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	Diluted hydrochloric 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="6">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>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1W</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent Concentrate (4×)/ RD5-26</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>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.
- Test tubes for dilution of standards and samples.

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 (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 × 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 (1×).

Plasma - Collect plasma using heparin or EDTA 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 (1×).

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

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Human serum and plasma samples recommend a 400-fold dilution. A suggested 400-fold dilution can be achieved by adding 20 µL of sample to 380 µL of Calibrator Diluent (1×). Complete the 400-fold dilution by adding 20 µL of the diluted sample to 380 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

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×).

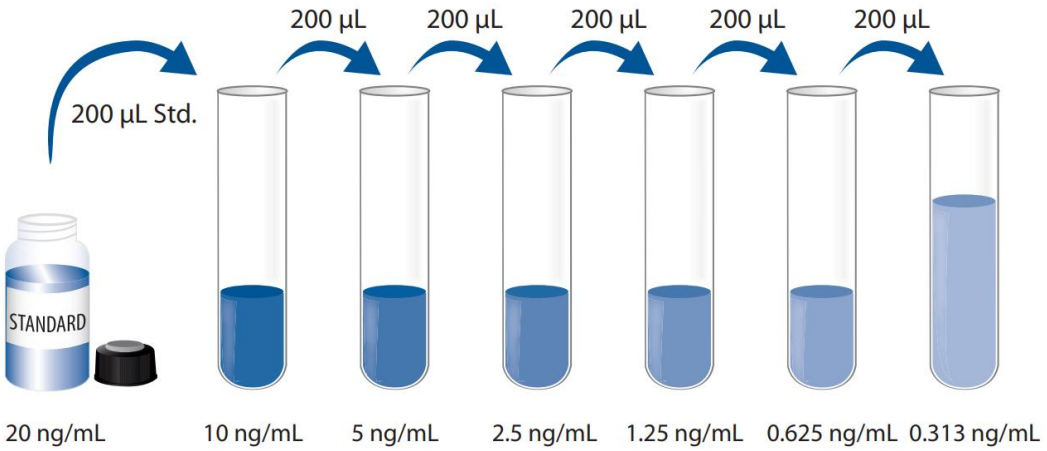
Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Human Complement Factor D Standard - Refer to the vial label for the

reconstitution volume*. Reconstitute the Human Complement Factor D Standard with Calibrator Diluent (1×). This reconstitution produces a stock solution of 20 ng/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 200 µL of Calibrator Diluent (1×) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human Complement Factor D Standard (20 ng/mL) serves as the high standard. Calibrator Diluent (1×) serves as the zero standard (0 ng/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.

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 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of standard and 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.
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 100 µL of Human Complement Factor D Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 100 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 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 Complement Factor D 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.

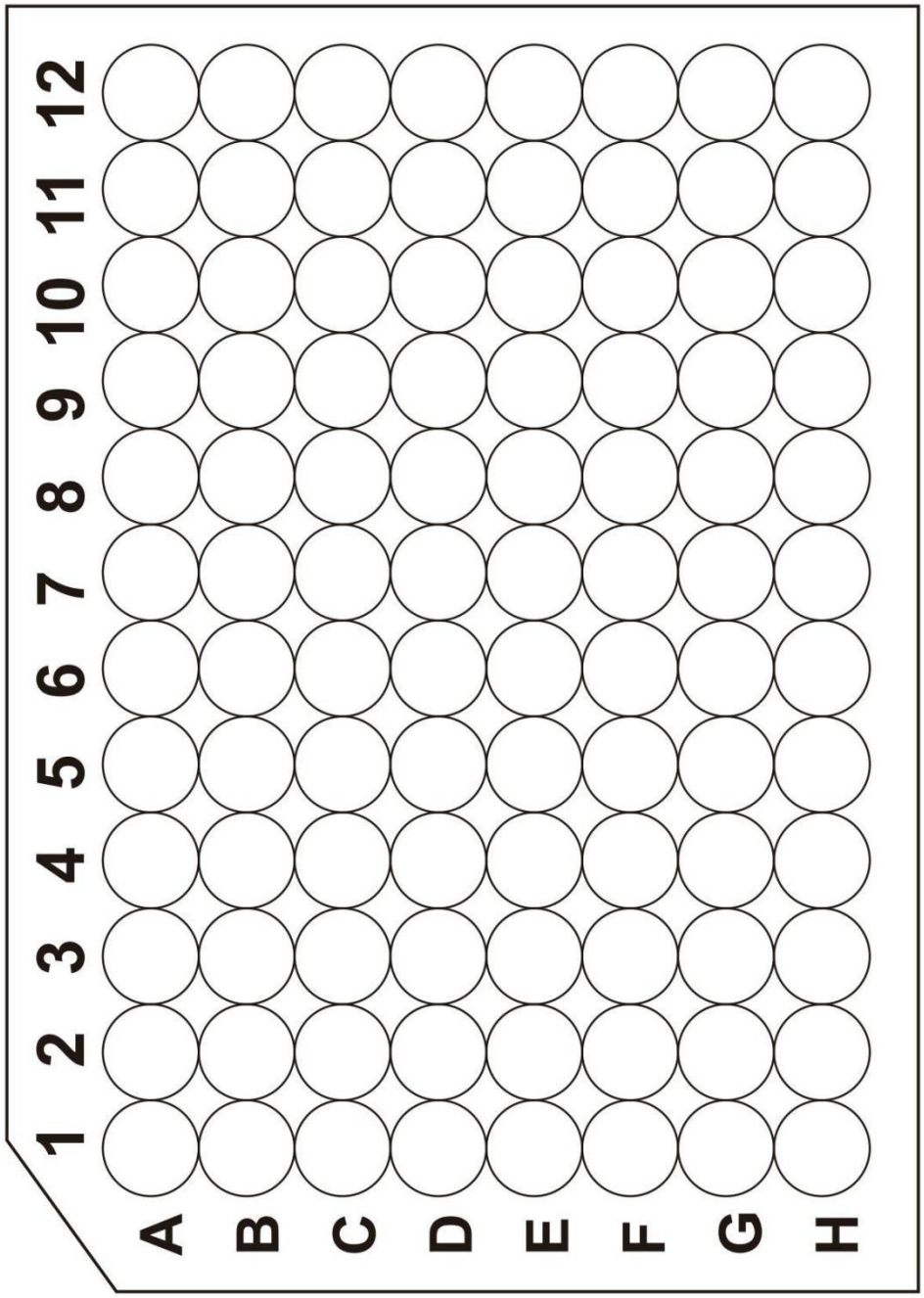
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Lesavre, P.H. *et al.* (1978) J. Exp. Med. 148:1498.
2. Volanakis, J.E. *et al.* (1996) Protein Sciences 5:553.
3. Cook, K.S. *et al.* (1985) Proc. Natl. Acad. Sci. USA 82:6480.
4. Flier, J.S. *et al.* (1987) Science 237:405.
5. Rosen, B.S. *et al.* (1989) Science 244:1483.
6. Xu, Y. *et al.* (2001) Proc. Natl. Acad. Sci. USA 98:14577.
7. Sanders, P.W. *et al.* (1986) J. Clin. Invest. 77:1299.
8. Volanakis, J.E. *et al.* (1985) N. Engl. J. Med. 312:395.
9. Biesma, D.H. *et al.* (2001) J. Clin. Invest. 108:233.
10. Sprong, T. *et al.* (2006) Blood. 107:4865.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Complement Factor D Valukine™ ELISA 试剂盒

目录号：VAL226

适用于定量检测天然和重组人 Complement Factor D 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

版本号 202411.1

目录

I. 背景

补体系统由大约 30 种循环血浆蛋白和细胞表面受体组成，作为先天性免疫系统和适应性免疫系统的一部分发挥着消除病原体的功能。该系统由多个蛋白水解级联组成，其中蛋白酶作为非活性酶原存在，并在上游活性蛋白酶的作用下被激活。补体激活有三种途径：经典途径、凝集素途径和替代途径。这些途径汇聚在C3转化酶的生成过程中，C3转化酶负责启动一系列事件，最终生成细菌疏松素（促进对疏松病原体的吞噬作用）、苞毒素（介导炎症）和末端膜攻击复合体的形成（诱导病原体或细胞的裂解）（1）。

补体因子D（factor D）又称adipsin，是一种丝氨酸蛋白酶，是通过替代途径启动补体激活所不可或缺的物质。Factor D通过底物诱导的可逆构象变化活化为活性酶，其功能是裂解与C3b结合的factor B，形成C3bBb复合物，即替代途径C3转化酶。人complement factor D以253个氨基酸（amino acid, aa）的前体形式合成，其中包含信号肽（aa 1-20）、五残基活化/前肽（aa 21-25）和成熟链（aa 26-253）。在生理条件下，缺乏活化肽的成熟factor D以非活性酶的形式循环，需要与其天然底物C3b结合的factor B相互作用，才能激活其催化活性（2）。成熟的人factor D与黑猩猩、恒河猴、猪和小鼠蛋白的 aa 序列同源性分别为98%、96%、84% 和66%。Factor D在多种组织中表达，包括单核细胞/巨噬细胞、肌肉、坐骨神经、子宫内膜、肾脏、肠道，在脂肪细胞中的表达量尤其高（3）。在各种肥胖症小鼠模型中，factor D的表达水平都有所降低（4-6）。

血清complement factor D的浓度通过肾脏中的分解代谢进行调节，factor D在肾小球中被过滤，并被近端肾小管重吸收（7）。在肾功能衰竭患者中，factor D的循环水平会升高。同样，在范可尼综合征（一种肾近曲小管功能受损的疾病）患者中，尿液中factor D的浓度也高度升高（8）。Complement factor D缺乏症与替代性补体途径的低活性和低溶菌能力有关。在factor D基因突变导致factor D完全缺乏的患者中，可以观察到反复的细菌感染（9, 10）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Complement Factor D抗体包被于微孔板上，样品和标准品中的人Complement Factor D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Complement Factor D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（1×）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。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值（ng/mL）	0.860	3.12	8.13	0.831	3.04	7.73
标准差	0.047	0.183	0.552	0.074	0.211	0.506
CV%	5.5	5.9	6.8	8.9	6.9	6.5

B. 回收率

不同类型样本中掺入检测范围内不同水平的人Complement Factor D，测定其回收率。

样本类型	平均回收率（%）	范围（%）
细胞培养基（n=4）	104	97-117
人尿液（n=4）	100	87-115

C. 灵敏度

评估了 40 次检测，人 Complement Factor D 的最小可检测剂量（MDD）范围为 0.005-0.025 ng/mL，平均 MDD 为 0.013 ng/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NS0表达的重组人Complement Factor D校正。

E. 线性

不同的样本中含有或掺入高浓度的人Complement Factor D，然后用标准品稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养上清液（n=4）	人血清*（n=4）	人EDTA血浆*（n=4）	人肝素血浆*（n=4）	人尿液（n=4）
1:2	平均值/期待值（%）	100	103	103	96	103
	范围（%）	98-106	99-110	95-106	90-101	97-110
1:4	平均值/期待值（%）	98	105	102	92	102
	范围（%）	89-113	94-112	89-115	91-94	96-104
1:8	平均值/期待值（%）	92	100	101	92	101
	范围（%）	87-100	89-111	90-111	90-96	96-105
1:16	平均值/期待值（%）	82	94	99	____	100
	范围（%）	____	85-103	93-105	____	99-100

*样品在分析前按照样品制备部分的要求进行稀释。

F. 样本预值

人血清/血浆/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Complement Factor D。本研究中使用的供体没有病史。

样本类型	平均值（ng/mL）	范围（ng/mL）	标准差（ng/mL）
人血清（n=35）	2339	100	1729-3238
人EDTA血浆（n=35）	2297	100	1468-3657
人肝素血浆（n=35）	1667	100	906-2545
人尿液（n=12）	1.83	75	ND-7.32

ND=未检出

细胞培养上清：

人外周血白细胞（1 × 10^6 cells/mL）在含有10%胎牛血清、5 μM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL链霉素硫酸盐的RPMI培养基中培养6天。取细胞培养上清液，测定人Complement Factor D的浓度，结果为13.2 ng/mL。

人外周血单核细胞（1 × 10^6 cells/mL）在含有10%胎牛血清、2 μM β-巯基乙醇、2 mML-谷氨酰胺、100 U/mL青霉素和100 μg/mL链霉素硫酸盐的RPMI培养基中培养。细胞在不刺激或用10 ng/mL重组人IL-2刺激1天后培养。取细胞培养上清液，测定人Complement Factor D的浓度。

条件	1 天（ng/mL）
未刺激	1.46
刺激	1.60

HT-29人结肠腺癌细胞（0.25 × 10^5 cells/mL）在含有10%胎牛血清、2 mML-谷氨酰胺、100 U/mL青霉素和100 μg/mL链霉素硫酸盐的McCoy's 5a培养基中培养7天。取细胞培养上清液一部分，测定人Complement Factor D的浓度，结果为0.798 ng/mL。

U937人组织细胞淋巴瘤细胞（1 × 10^5 cells/mL）在含有10%胎牛血清和2 mM L-谷氨酰胺的RPMI中培养1天和3天。取细胞培养上清液，测定人Complement Factor D的浓度。

条件	1 天（ng/mL）	3 天（ng/mL）
未刺激	3.17	5.61

树突状细胞（1 × 10^6 cells/mL）在含有10%胎牛血清、5 μM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL链霉素硫酸盐的RPMI培养基中培养。细胞用1000 U/mL重组人GM-CSF和500 U/mL重组人IL-4刺激7天。取细胞培养上清液，测定人Complement Factor D的浓度，结果为2.89 ng/mL。

G. 特异性

检测方法识别天然和重组人Complement Factor D。

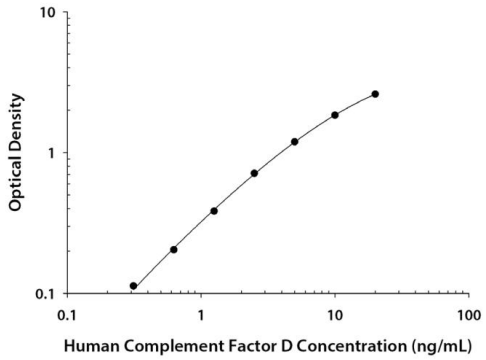
以下列出的因子在标准品稀释液（1×）中以200 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人Complement Factor D对照品中以200 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human:	**Natural proteins:**
Coagulation Factor II/Thrombin	bovine Coagulation Factor Xa
Coagulation Factor VII	human Coagulation Factor II/Thrombin
Coagulation Factor X	
Coagulation Factor Xa	
Coagulation Factor XI	
Complement Component C1r	
Complement Component C1s	
Complement Component C2	
Complement Component C3	
Complement Factor B	
Complement Factor H-related 1/CFHR1	
Complement Factor H-related 5/CFHR5	

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。



(ng/mL)	O.D.	Average	Corrected
0	0.015 0.017	0.016	—
0.313	0.125 0.132	0.129	0.113
0.625	0.217 0.223	0.220	0.204
1.25	0.394 0.406	0.400	0.384
2.50	0.720 0.736	0.728	0.712
5.00	1.200 1.210	1.205	1.189
10.0	1.840 1.880	1.860	1.844
20.0	2.590 2.650	2.620	2.604

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Complement Factor D Microplate	包被抗人Complement Factor D抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human Complement Factor D Conjugate	酶标检测抗人Complement Factor D抗体	1瓶
Human Complement Factor D Standard	重组人Complement Factor D标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1W	检测液	1瓶
Calibrator Diluent Concentrate (4×)/ RD5-26	浓缩的标准品稀释液（4×），用于稀释标准品和样品	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

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="7">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1W</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（4×）/ RD5-26</td><td>2-8°C 储存，最多 30 天*
请每次使用新鲜配制的1×标准品稀释液，多余的丢弃</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°C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
- 100 mL和500 mL量筒
- 用于稀释标准品和样品的管子

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（1×）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。样本可能需要用标准品稀释液（1×）稀释。

血浆 - 使用肝素或EDTA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（1×）稀释。

注：*柠檬酸盐血浆在本检测中未经验证。*

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。样品可能需要用标准品稀释液（1×）进行稀释。

B. 样品准备

人血清和人血浆样本建议稀释 400 倍。建议的 400 倍稀释方式为：将 20 µL 样品加到 380 µL 标准品稀释液（1×）中，再将 20 µL 已稀释样本加入 380 µL 标准品稀释液（1×）中，完成 400 倍稀释。最佳稀释倍数应由用户自行确定。

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（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×）。

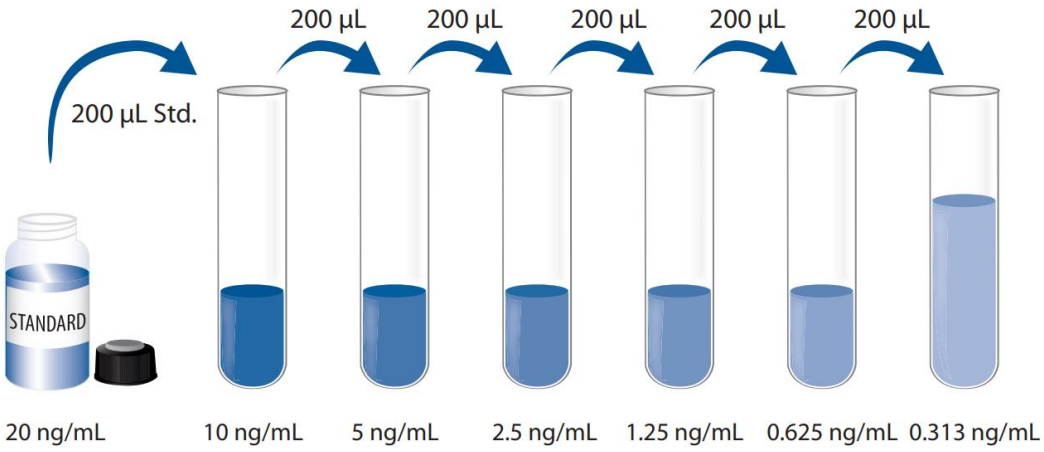
标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人Complement Factor D标准品：复溶体积请参考瓶身标签*，用标准品稀释液（1×）复溶人Complement Factor D标准品，得到浓度为20 ng/mL标准品储备母液。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。

用移液管将200 µL标准品稀释液（1×）移入每个管中，使用标准品储备母液制备2倍稀

释梯度（如下图所示）。在每次转移之前，将每个管子彻底混合。未稀释的人Complement Factor D标准品（20 ng/mL）作为标准曲线的最高点。标准品稀释液（1×）作为标准曲线的零点（0 ng/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放置于室温。建议对所有标准品和样品进行复孔检测。

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. 向每个孔中加入50 µL的检测液RD1W。
4. 分别将不同浓度标准品和实验样本加入相应孔中，每孔50 µL。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。
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100 µL人Complement Factor D酶标检测抗体。用新的封板膜封住反应孔，**室温孵育1小时**。
7. 重复第5步洗板操作。
8. 在每个微孔内加入100 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100 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.），使用计算机软件作四参数逻辑（4-PL）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轴上的浓度来建立标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人Complement Factor D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。该程序将产生足够但不太精确的数据拟合。

    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. Lesavre, P.H. *et al.* (1978) J. Exp. Med. 148:1498.
2. Volanakis, J.E. *et al.* (1996) Protein Sciences 5:553.
3. Cook, K.S. *et al.* (1985) Proc. Natl. Acad. Sci. USA 82:6480.
4. Flier, J.S. *et al.* (1987) Science 237:405.
5. Rosen, B.S. *et al.* (1989) Science 244:1483.
6. Xu, Y. *et al.* (2001) Proc. Natl. Acad. Sci. USA 98:14577.
7. Sanders, P.W. *et al.* (1986) J. Clin. Invest. 77:1299.
8. Volanakis, J.E. *et al.* (1985) N. Engl. J. Med. 312:395.
9. Biesma, D.H. *et al.* (2001) J. Clin. Invest. 108:233.
10. Sprong, T. *et al.* (2006) Blood. 107:4865.

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