



PRODUCT INFORMATION & MANUAL

Human Fibronectin Valukine™ ELISA

VAL179

For the quantitative determination of natural and recombinant human Fibronectin 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

Version202211.1

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I. BACKGROUND

Fibronectin (FN) is a large modular glycoprotein that is found as a polymeric fibrillar network in the extracellular matrix (ECM) and as soluble disulfide-linked dimeric protomers in plasma and other body fluids (1-4). Fibronectin is a ligand for many molecules including fibrin, heparin, chondroitin sulfate, collagen/gelatin, various integrins and growth factors, myocilin, and Apolipoprotein A (4-10). It is involved in multiple cellular processes such as cell adhesion/ migration, blood clotting, morphogenesis, tissue repair, and cell signaling. Fibronectin functions are mediated by the insoluble polymeric fibrils. Conversion of soluble Fibronectin to Fibronectin fibrils in the ECM is initiated by binding to cell surface integrins, resulting in exposure of cryptic epitopes necessary for polymerization (4). Mature human Fibronectin shares 88% aa sequence identity with mouse and rat Fibronectin.

Fibronectin is made up of three types of homologous structural motifs termed FN type I, type II, and type III repeats (11-13). Alternative splicing generates multiple isoforms of Fibronectin which may have insertions of extra type III domains (EDA and EDB) or alteration of the type III connecting segment (IIICS) (3, 13). Differential splicing within the IIICS domain determines the presence of CS1 and CS2 sequences and the sensitivity to proteases (14, 15). The IIICS domain contains two sites (CS1 and CS2) that interact with Integrin $\alpha4\beta1$ (14, 16, 17). The CS1 sequence is not accessible in full length Fibronectin but is exposed by protease digestion, thereby enabling cell adhesion via Integrin $\alpha4\beta1$ (17). A fragment from the first type III repeat, known as Anastellin, binds to Fibronectin and induces the formation of high molecular weight disulfide-linked superfibronectin (sFN) (18-20). sFN exhibits increased protease sensitivity and cellular adhesiveness compared to Fibronectin (18, 20). Anastellin acts in vivo to inhibit angiogenesis, tumor growth, and metastasis (19).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Fibronectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Fibronectin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human Fibronectin 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 is added to the wells and color develops in proportion to the amount of human Fibronectin 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 (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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
Mean (ng/mL)	1.6	5.8	24.1	1.5	5.8	24.1
Standard Deviation	0.1	0.2	1.5	0.1	0.2	1.4
CV%	3.3	3.4	6.2	4.7	3.4	5.7

B. RECOVERY

The recovery of human Fibronectin spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 82.8-121.1% with an average of 101.8%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human Fibronectin is typically less than 0.011 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 human Fibronectin purified from human plasma at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Fibronectin 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	99.9	91.6-109.2
1:4	103.7	85.9-119.7
1:8	104.4	85.9-123.9
1:16	106.3	87.7-121.8

F. SAMPLE VALUES

Cell Culture Supernates - MCF-7 cells were seeded in 100 mL of DMEM/F12 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 2 days. The cell culture supernate was assayed for human Fibronectin and measured 18.5 ng/mL.

TF-1 cells were seeded in 100 mL of 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate, 20ng/mL GM-CSF for 2 days. The cell culture supernate was assayed for human Fibronectin and measured 4 ng/mL.

Serum - Six human serum samples were evaluated for the presence of human Fibronectin in this assay. All samples measured ranged from 14.6 to 117.0 µg/mL with an average of 54.9 µg/mL.

G. SPECIFICITY

The following factors prepared at 2000 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	
COMP	Integrin αVβ1
Fibulin 1C	Integrin αVβ3
Galectin-3BP	Integrin αVβ6
Integrin α4β7	Integrin αVβ8
Integrin α5β1	Tenascin R

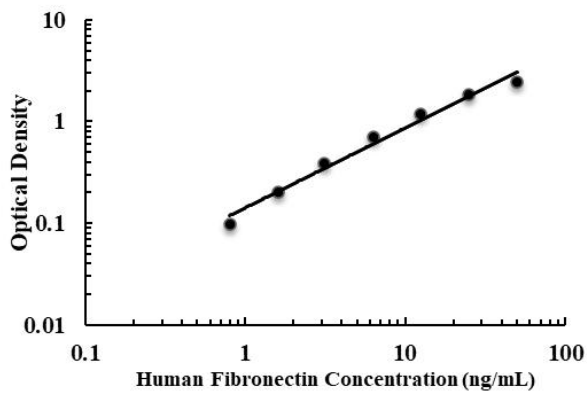
A sample containing 250 ng/mL of recombinant human Fibronectin (aa 631-705) reads as 4 ng/mL (1.6% cross-reactivity).

A sample containing 1000 ng/mL of bovine Fibronectin reads as 4 ng/mL (0.4% cross-reactivity). Bovine Fibronectin may be present in Bovine Sera.

IV. EXPERIMENT

EXAMPLE STANDARD

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



ng/mL	OD	Average	Corrected
0	0.029 0.029	0.029	-
0.8	0.126 0.129	0.128	0.099
1.6	0.227 0.232	0.230	0.201
3.1	0.411 0.414	0.413	0.384
6.3	0.733 0.736	0.735	0.706
12.5	1.205 1.227	1.216	1.187
25	1.856 1.872	1.864	1.835
50	2.445 2.458	2.452	2.423

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Fibronectin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human Fibronectin.	1 plate
Human Fibronectin Standard	Human Fibronectin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human Fibronectin Detection Antibody	Biotinylated human Fibronectin antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent Concentrate (2×)	Concentrated buffered diluent used to dilute standard and samples.	2 vials
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

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP A	May be stored for up to 1 month at 2-8 °C.*
	Diluted Wash Solution	
	TMB Substrate	
	Stop Solution	
	Standard	Prepare fresh for each assay. Standards may be stored for up 1 month at -20°C.*
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *
	Reagent Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.
	Calibrator Diluent Concentrate (2×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.
Microplate Wells	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.*	

* 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.
- ◆ The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.
- ◆ Human Fibronectin is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1 \times).

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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1 \times).

B. SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution prior to the assay. A suggested 2-fold dilution is 100 μL of sample + 100 μL of Calibrator Diluent (1 \times).

Human serum samples require a 2000-fold dilution. For example, add 10 μL of serum into a tube with 90 μL Calibrator Diluent (1 \times) to prepare a 10-fold diluted sample. Mix through and then pipette 10 μL of prepared 10-fold diluted sample into a tube with 90 μL Calibrator Diluent (1 \times) to prepare a 100-fold diluted sample. Mix through and then pipette 10 μL of prepared 100-fold diluted sample into a tube with 190 μL Calibrator Diluent (1 \times) to prepare a 2000-fold diluted sample.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: Human Fibronectin are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

Wash Buffer (1 \times) - 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 \times) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 \times).

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

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

Detection Antibody - **Centrifuge briefly before opening. Reconstitution Volume refer to vial label with Reagent Diluent (1 \times).** Aliquot and store if needed. Dilute stock solution in Reagent Diluent (1 \times) to the working concentration of 12.5 ng/mL. Prepare at least 15 minutes prior to use.

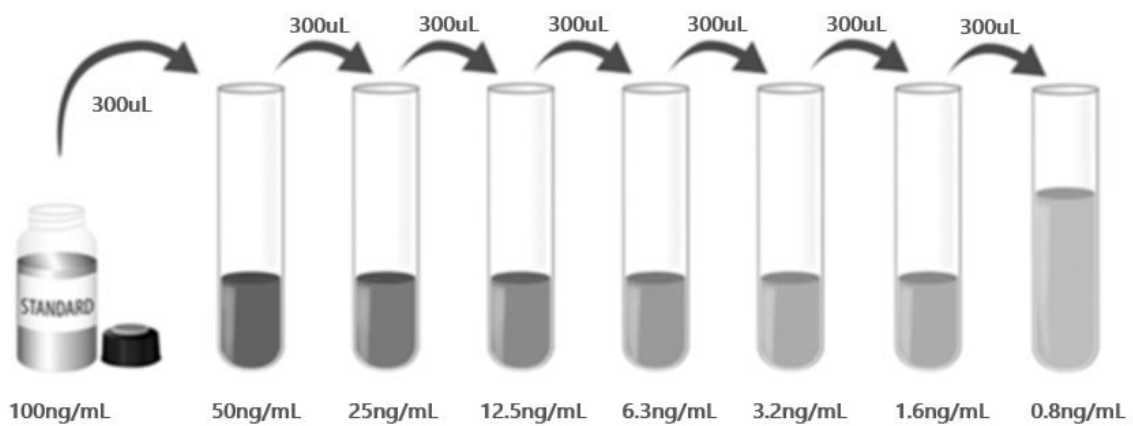
Streptavidin-HRP A (1 \times) - **Centrifuge briefly before opening.** Dilute to the working

concentration specified on the vial label using Reagent Diluent (1×).

Human Fibronectin Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 100 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 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 50 ng/mL standard serves as the high standard. The Calibrator Diluent (1×) 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 reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: Human Fibronectin are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

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 μ L 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 μ L) 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 μ L of the Detection Antibody diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate for 2 hours at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μ L of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 20 minutes at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μ L of TMB Substrate to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
10. Add 50 μ L of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. 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.

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 human Fibronectin 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.

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



产品信息及操作手册

人 Fibronectin Valukine™ ELISA 试剂盒

目录号: VAL179

适用于定量检测天然和重组人 Fibronectin 的浓度

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I. 背景

纤维连接蛋白(FN)是一种大型模块化糖蛋白,在细胞外基质(ECM)中作为聚合纤维网络存在,在血浆和其他体液中作为可溶性二硫键二聚体存在(1-4)。纤维结合蛋白是许多分子的配体,包括纤维蛋白、肝素、硫酸软骨素、胶原蛋白/明胶、各种整合素和生长因子、心肌素和载脂蛋白A (4-10)。它参与多种细胞过程,如细胞粘附/迁移、凝血、形态发生、组织修复和细胞信号传导。纤维连接蛋白的功能由不溶性聚合物纤维介导。ECM中可溶性纤维结合蛋白转化为纤维结合蛋白原纤维是通过与细胞表面整合素结合,导致聚合所需的隐性表位暴露(4)。成熟的人纤维结合蛋白与小鼠和大鼠纤维结合蛋白具有88%的氨基酸序列同源性。

纤维连接蛋白由三种类型的同源结构基序组成,称为FN I型、II型和III型重复序列(11-13)。选择性剪接产生多种纤维连接蛋白异构体,这些异构体可能有额外的III型结构域(EDA和EDB)插入或III型连接段(IIICS)改变(3, 13)。IIICS结构域内的差异剪接决定了CS1和CS2序列的存在以及对蛋白酶的敏感性(14, 15)。IIICS结构域包含两个位点(CS1和CS2),它们与整合素 $\alpha 4\beta 1$ 相互作用(14, 16, 17)。CS1序列在全长的纤维连接蛋白中不可获得,但通过蛋白酶消化暴露,从而通过整合素 $\alpha 4\beta 1$ 实现细胞粘附(17)。来自第一个III型重复的片段,称为Anastellin,与纤维连接蛋白结合,并诱导形成高分子量二硫键连接超纤维连接蛋白(sFN) (18-20)。与纤维连接蛋白相比,sFN表现出更高的蛋白酶敏感性和细胞粘附性(18, 20)。Anastellin在体内可以抑制血管生成、肿瘤生长和转移(19)。

II. 概述

A. 检测原理

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

样本	板内精确度			板间精确度		
	1	2	3	1	2	3
平均值 (pg/mL)	1.6	5.8	24.1	1.5	5.8	24.1
标准差	0.1	0.2	1.5	0.1	0.2	1.4
CV%	3.3	3.4	6.2	4.7	3.4	5.7

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Fibronectin，测定其回收率。回收率范围在82.8-121.1%，平均回收率在101.8%。

C. 灵敏度

人Fibronectin的最低可测剂量（MDD）一般小于0.011 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

此ELISA试剂盒从人血浆中纯化的人纤维连接蛋白进行校准。

E. 线性

不同的样本中含有或掺入高浓度的人Fibronectin，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	平均值 (%)	范围 (%)
1:2	99.9	91.6-109.2
1:4	103.7	85.9-119.7
1:8	104.4	85.9-123.9
1:16	106.3	87.7-121.8

F. 样本预值

细胞培养上清液 - MCF-7培养于100 mL的DMEM/F12培养基中，细胞培养基还含有10%胎牛血清，100 U/mL青霉素和100 µg/mL硫酸链霉素培养两天。取细胞培养上清测定人Fibronectin含量，结果为18.5 ng/mL。

TF-1培养于100 mL的1640培养基中，细胞培养基还含有10%胎牛血清，100 U/mL青霉素和100 µg/mL硫酸链霉素，20 ng/mL GM-CSF培养两天。取细胞培养上清测定人Fibronectin含量，结果为4 ng/mL。

血清样本 - 使用本试剂盒检测了6份人血清样本中Fibronectin的水平。6份样本的检测值在14.6-117.0 µg/mL之间，平均值为54.9 µg/mL。

G. 特异性

将以下因子配制成2000 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:	
COMP	Integrin αVβ1
Fibulin 1C	Integrin αVβ3
Galectin-3BP	Integrin αVβ6
Integrin α4β7	Integrin αVβ8
Integrin α5β1	Tenascin R

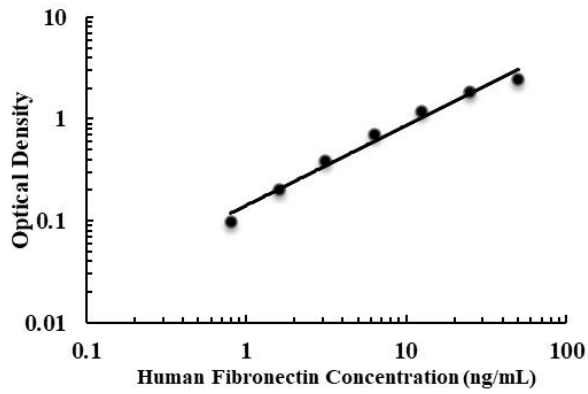
含有250 ng/mL重组人纤维连接蛋白（631-705 aa）的样品读数为4 ng/mL（1.6%交叉反应性）。

含有1000 ng/mL牛纤维连接蛋白的样品读数为4 ng/mL（0.4%交叉反应性）。牛纤维连接蛋白可能存在于牛血清中。

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	OD	Average	Corrected
0	0.029	0.029	-
0.8	0.126 0.129	0.128	0.099
1.6	0.227 0.232	0.230	0.201
3.1	0.411 0.414	0.413	0.384
6.3	0.733 0.736	0.735	0.706
12.5	1.205 1.227	1.216	1.187
25	1.856 1.872	1.864	1.835
50	2.445 2.458	2.452	2.423

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Fibronectin Microplate	包被小鼠抗人 Fibronectin 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human Fibronectin Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human Fibronectin Detection antibody	生物素化的人 Fibronectin 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent Concentrate (2×)	浓缩的标准品稀释液（2×）	2 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×）	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

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	链霉亲和素-HRP A	2-8℃储存，最多 30 天*
	洗涤缓冲液（1×）	
	TMB 底物溶液	
	终止液	
	标准品	使用时新鲜配制* 标准品-20℃储存，最多 30 天*
	检测抗体	分装， -20℃储存，最多 30 天*
	试剂稀释液（10×）	2-8℃储存，最多 30 天* 请每次使用新鲜配制的 1×试剂稀释液
	标准品稀释液（2×）	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
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
- ◆ 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、面部及衣服的保护。使用后请彻底洗手。
- ◆ 唾液中含有人Fibronectin，为防止试剂盒在检测过程中产生污染，请采取防护措施。

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（1×）稀释。

B. 样本准备工作

细胞培养上清样本需要用标准品稀释液（1×）2倍稀释后进行检测，例如：

100 μL样本+100 μL标准品稀释液（1×）。

人血清样本需要2000倍稀释后进行检测。例如：

10 μL血清加到90 μL标准品稀释液（1×）中，充分混匀，即样本10倍稀释。然后取10 μL 10倍稀释后的样本加到90 μL标准品稀释液（1×）中，充分混匀，即样本100倍稀释。然后取10 μL 100倍稀释后的样本加到190 μL标准品稀释液（1×）中，充分混匀，即制备成2000倍稀释的样本。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

注：唾液中含有人 *Fibronectin*，为避免污染，实验时请戴口罩、手套。

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稀释配制成标准品稀释液（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×）。

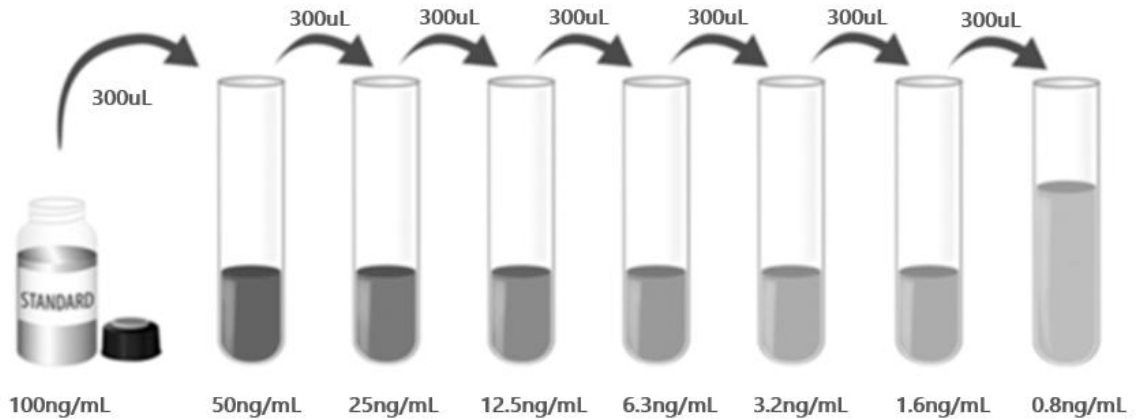
检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶。再用试剂稀释液（1×）稀释至工作浓度12.5 ng/mL，至少在使用前15分钟准备。

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Fibronectin 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100 ng/mL标准品母液。轻轻震荡至少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 μ L标准品稀释液（1 \times ）。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。50 ng/mL管作标准曲线最高点，标准品稀释液（1 \times ）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。

注：唾液中含有人Fibronectin，为避免污染，实验时请戴口罩、手套。

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100 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；（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 配制好的检测抗体。用封板膜封住反应孔，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100 μL TMB底物溶液，室温孵育20分钟。**注意避光；**
10. 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。孔内溶液颜色会从蓝色变为黄色；
11. 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；
12. **计算结果：**将每个标准品和样品的校正吸光度值（ $\text{OD}_{450}-\text{OD}_{540}/\text{OD}_{570}$ ），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Fibronectin 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

