PRODUCT INFORMATION & MANUAL

Human FGF-21 Valukine™ ELISA

Catalog Number: VAL207

For the quantitative determination of natural and recombinant human Fibroblast Growth Factor 21 (FGF-21) 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 202410.1

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I. BACKGROUND

Fibroblast growth factor 21 (FGF-21) is a member of the FGF gene family, which contains 22 mammalian members. Based on its structure, it is further classified as a member of the FGF-19 subfamily, which also includes FGF-19 and FGF-23 (1-4). FGF family members contain a 120 amino acid (aa) core FGF domain that exhibits a β-trefoil structure. FGF-19 subfamily members, unlike other FGFs, lack one strand of the β-trefoil and bind poorly to extracellular matrix molecules such as heparin (3). They are consequently more diffusible than other FGFs and are considered endocrine rather than paracrine (1-4). All three subfamily members impact some aspect of metabolism; all three are induced by a nuclear receptor heterodimer that includes RXR (retinoid X receptor), and all three bind FGF receptors (FGF R) indirectly through co-receptors of the klotho family (5-9). FGF-21 binds to β-Klotho via its C-terminal sequence. This binding, along with amino acids at the N-terminus, is required for signaling through FGF R (7, 8). FGF-21 is selective for FGF R1 isoform 1c, with varying reports of using isoforms 2c or 3c (10-12). Presence of the required klotho and FGF R family members determines tissue specificity of FGF-19 subfamily members, and thus concentrates FGF-21 activity within adipose tissue (3, 9-11). Mature human FGF-21 shares 81% aa sequence identity with mouse and rat FGF-21.

FGF-21 is produced by hepatocytes in response to free fatty acid (FFA) stimulation of a PPARα/RXR dimeric complex (4, 13-15). This situation occurs during starvation, diabetic ketosis, or following the ingestion of a high-fat/low-carbohydrate or ketogenic diet (5, 14-16). Upon FGF-21 secretion, white adipose tissue is induced to release FFAs from triglyceride stores. Once FFAs reach the hepatocytes, they are oxidized and reduced to acetyl-CoA (16). The acetyl-CoA is recombined into 4-carbon ketone bodies (acetoacetate and β-hydroxybutyrate), released, and transported to peripheral tissues for energy generation (5, 15, 16).

FGF-21 production is also induced upon differentiation of human or mouse fibroblasts to adipocytes (17, 18). In adipose tissue, FGF-21 induces glucose uptake by signaling in synergy with PPARγ to increase production of the glucose transporter, GLUT1 (10, 12, 19). FGF-21 production follows a circadian pattern in mice (20). It diffuses across the blood-brain barrier and this may facilitate induction of a state of torpor, or

decreased activity, in response to increased FGF-21 (16, 21). These characteristics appear to induce a hibernation-like state during fasting and short days in winter (22). In diet-induced obese mice and mouse models of diabetes such as db/db and ob/ob, administration or transgenic overexpression of FGF-21 restores circulating glucose and triglyceride values to near normal and increases insulin sensitivity (5, 6, 14, 23, 24). In some of these states and in human obesity and type II diabetes, FGF-21 is already elevated prior to treatment, suggesting that resistance to FGF-21 is possible (17, 25, 26). Although FGF-21 administration corrects obesity in mice, it is unclear whether the same benefit would be seen in humans (2, 3, 17, 26-28).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human FGF-21 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human FGF-21 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human FGF-21 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 FGF-21 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 and human plasma.
- 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 (pg/mL)	204	672	1324	245	742	1411
Standard Deviation	8.0	19.8	46.2	26.6	47.2	73.9
CV%	3.9	2.9	3.5	10.9	6.4	5.2

B. RECOVERY

The recovery of human FGF-21 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=4)	98	93-108
Human Serum (n=4)	105	87-119
Human Heparin Plasma (n=4)	98	83-111
Human EDTA Plasma (n=4)	103	92-112

C. SENSITIVITY

Fifty-five assays were evaluated and the minimum detectable dose (MDD) of human FGF-21 ranged from 1.61-8.69 pg/mL. The mean MDD was 4.67 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 FGF-21 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 FGF-21 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Human Serum (n=4)	Human EDTA plasma (n=4)	Human Heparin plasma (n=4)
1:2	Average % of Expected	105	101	101	102
	Range (%)	103-107	96-108	99-103	97-105
1:4	Average % of Expected	105	98	102	104
	Range (%)	102-107	96-103	98-104	99-110
1:8	Average % of Expected	101	98	100	101
	Range (%)	99-103	94-102	98-101	97-104
1:16	Average % of Expected	95	90	93	101
	Range (%)	91-104	86-94	90-97	99-105

F. SAMPLE VALUES

Human Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human FGF-21 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Human Serum (n=35)	172	97	ND-914
Human EDTA plasma (n=35)	201	97	ND-1155
Human Heparin plasma (n=35)	186	97	ND-1012

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood leukocytes (PBLs) were cultured in DMEM supplemented with 5% fetal bovine serum, 5 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 µg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for human FGF-21. No detectable levels were observed.

G. SPECIFICITY

This assay recognizes natural and recombinant human FGF-21.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human FGF-21 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:			**Recombinant mouse:**
β-ECGF	Flt-3/Flk-2 Ligand	MSPβ	FGF-8b
EGF	Flt-4	MSP R	FGF-8c
EGF (pro)	G-CSF	NGF R	FGF-23
EGF R	G-CSF R	β-NGF	FGF basic
EG-VEGF	GM-CSF	PD-ECGF	FGF R2β
FGF-3	HB-EGF	PDGF-AA	FGF R3
FGF-4	HGF	PDGF-AB	Klotho
FGF-5	HGF Activator	PDGF-BB	**Recombinant rat:**
FGF-6	HGF R	PDGF-CC	FGF basic

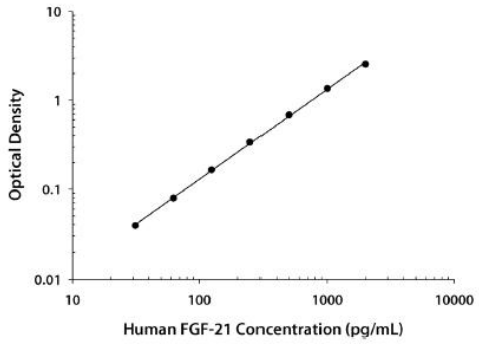
FGF-9	HRG-α	PDGF-DD	FGF-BP
FGF-10	IGFBP-1	PDGF Rα	**Recombinant canine:**
FGF-12	IGFBP-2	PDGF Rβ	KGF/FGF-7
FGF-16	IGFBP-3	PlGF	**Natural Proteins:**
FGF-17	IGFBP-4	PPARα	bovine FGF acidic
FGF-18	IGFBP-5	VEGF121	bovine FGF basic
FGF-19	IGFBP-6	VEGF162	human PDGF
FGF-20	IGF-I	VEGF165	porcine PDGF
FGF-22	IGF-I R	VEGF-B167	
FGF-23	IGF-II	VEGF-C (Cys156Ser)	
FGF acidic	IGF-II R	VEGF-C (wild type)	
FGF basic	IGFBP-rp1	VEGF-D	
FGF R1α	KGF/FGF-7	VEGF/PlGF	
FGF R2α	Klotho	VEGF R1	
FGF R2β	β-Klotho	VEGF R2	
FGF R3	M-CSF	VEGF R3	
FGF R4	M-CSF R		
Flt-3	MSP		

Recombinant mouse FGF-21 cross-reacts approximately 21% in this assay.

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)	O.D.	Average	Corrected
0	0.034 0.034	0.034	—
31.3	0.072 0.074	0.073	0.039
62.5	0.111 0.114	0.113	0.079
125	0.198 0.200	0.199	0.165
250	0.361 0.384	0.373	0.339
500	0.708 0.728	0.718	0.684
1000	1.368 1.409	1.389	1.355
2000	2.549 2.594	2.572	2.538

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human FGF-21 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human FGF-21	1 plate
Human FGF-21 Conjugate	Solution of antibody against human FGF-21 conjugated to horseradish peroxidase	1 vial
Human FGF-21 Standard	Recombinant human FGF-21 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	1 vial
Assay Diluent RD1S	A buffered protein base	1 vial
Calibrator Diluent Concentrate (2×)/ RD6-10	A 2× 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	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="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
RD1S</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent
Concentrate (2×)/
RD6-10</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.

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.*

B. 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×).

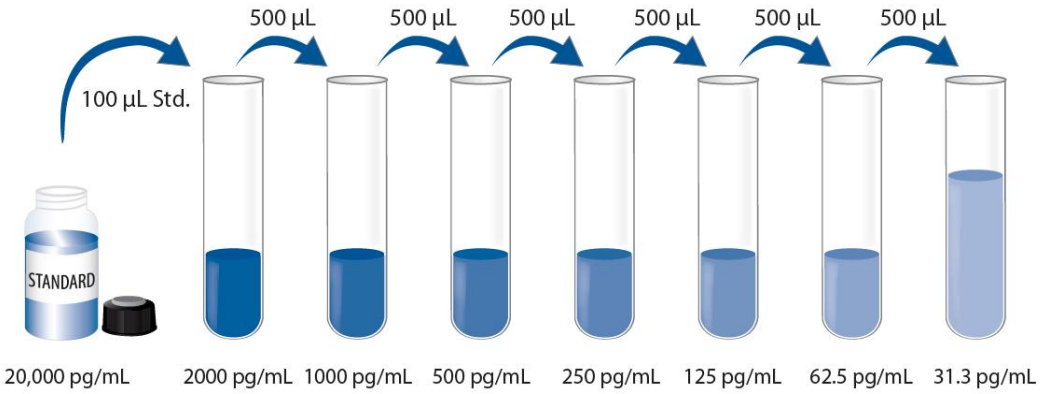
Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Human FGF-21 Standard - Refer to the vial label for the reconstitution volume*
Reconstitute the Human FGF-21 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20000 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 (1×) into the 2000

pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



C. 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 100 µL of Assay Diluent RD1S 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 200 µL of Human FGF-21 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 FGF-21 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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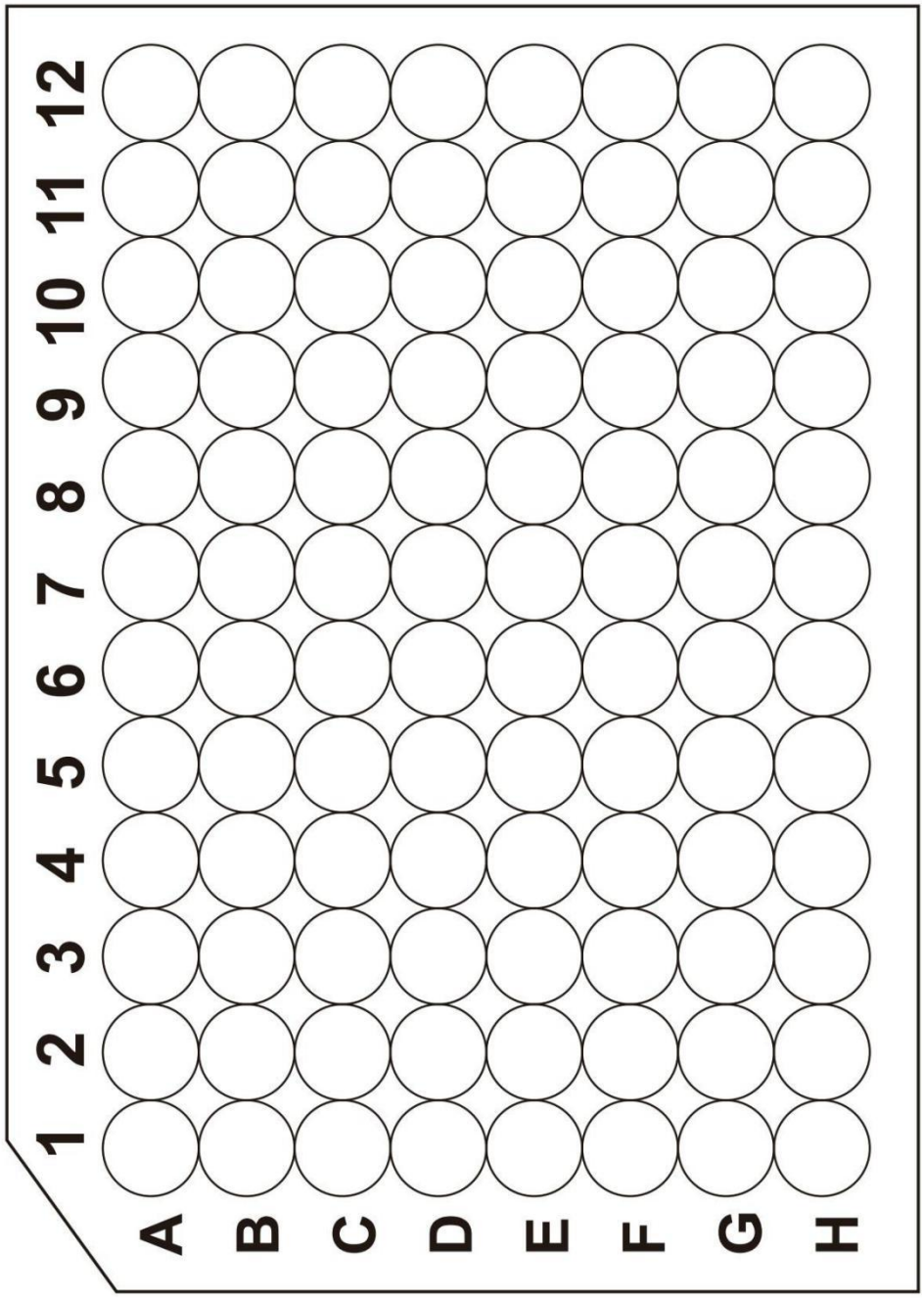
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 FGF-21 Valukine™ ELISA 试剂盒

目录号：VAL207

适用于定量检测天然和重组人成纤维细胞生长因子 21（FGF-21）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

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版本号 202410.1

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I. 背景

成纤维细胞生长因子 21（Fibroblast growth factor 21 , FGF-21）是 FGF 基因家族的一个成员，该基因家族包含 22 个哺乳动物成员。根据其结构，它又被归类为 FGF-19 亚家族成员，该亚家族还包括 FGF-19 和 FGF-23 (1-4)。FGF 家族成员包含一个 120 氨基酸（amino acid ,aa）的核心 FGF 结构域，该结构域呈β-三叶形结构。与其他 FGF 不同的是，FGF-19 亚家族成员缺少β-三叶草结构的一条链，与肝素等细胞外基质分子的结合力较差 (3)。它们比其他成纤维细胞生长因子更易扩散，被认为是内分泌性的，而不是旁分泌性的（1-4）。所有三个亚家族成员都会影响新陈代谢的某些方面；所有三个亚家族成员都会受到包括类视黄醇 X 受体（retinoid X receptor, RXR）在内的核受体异二聚体的诱导，并且所有三个亚家族成员都会通过 klotho 家族的共受体间接结合 FGF 受体（FGF R）（FGF receptors，FGF R）（5-9）。FGF-21 通过其 C 端序列与 β-Klotho 结合。这种结合以及 N 端的氨基酸是通过 FGF R 信号转导（7，8）。FGF-21 对 FGF R1 受体亚型 1c 有特异性，关于使用 2c 或 3c 亚型的报告则有所不同（10-12）。所需 klotho 和 FGF R 家族成员的存在决定了 FGF-19 亚家族成员的组织特异性，从而使 FGF-21 的活性集中在脂肪组织内（3，9-11）。成熟的人 FGF-21 与小鼠和大鼠 FGF-21 有 81%的 aa 序列相同性。

肝细胞在游离脂肪酸（free fatty acid，FFA）刺激 PPARα/RXR 二聚体复合物时会产生 FGF-21（4，13-15）。这种情况发生在饥饿、糖尿病酮症或摄入高脂肪/低碳水化合物或生酮饮食后（5，14-16）。FGF-21 分泌后，会诱导白色脂肪组织从甘油三酯储存库中释放出 FFAs。一旦 FFAs 到达肝细胞，就会被氧化并还原成乙酰辅酶 A（16）。乙酰辅酶 A 被重新组合成 4 -碳酮体（乙酰乙酸酯和 β-羟基丁酸），释放出来并输送到外周组织用于产生能量（5，15，16）。

人或小鼠成纤维细胞分化为脂肪细胞时，也会诱导 FGF-21 的产生（17，18）。在脂肪组织中，FGF-21 通过与 PPARγ协同传递信号，增加葡萄糖转运体 GLUT1 的生成，从而诱导葡萄糖摄取（10，12，19）。FGF-21 的产生遵循小鼠的昼夜节律（20）。它可以通过血脑屏障扩散，这可能有助于诱导倦怠状态或活动减少，以应对 FGF-21 的增加（16，21）。在冬季禁食和日照时间短的情况下，这些特征似乎会诱发类似冬眠的状态（22）。在饮食诱导的肥胖小鼠和糖尿病小鼠模型，如 db/db 和 ob/ob 中，服用或转基因过表达 FGF-21 可使循环血糖和甘油三酯值恢复到接近正常值，并提高胰岛素敏感性（5，6，14，23，24。）在其中一些状态以及人类肥胖症和 II 型糖尿病中，治疗前 FGF-21 已经升高，这表明 FGF-21 可能具有抗药性（17，25，26）。虽然服用 FGF-21 能纠正小鼠的肥胖症，但目前还不清楚人类是否也能从中获益（2，3，17，26-28）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人FGF-21抗体包被于微孔板上，样品和标准品中的人FGF-21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FGF-21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样本，人血清和人血浆；
- 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 (pg/mL)	204	672	1324	245	742	1411
标准差	8.0	19.8	46.2	26.6	47.2	73.9
CV%	3.9	2.9	3.5	10.9	6.4	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FGF-21，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=4)	98	93-108
人血清 (n=4)	105	87-119
人肝素血浆 (n=4)	98	83-111
人 EDTA 血浆 (n=4)	103	92-112

C. 灵敏度

进行55次检测评估，人FGF-21的最低可检测剂量（MDD）范围为1.61-8.69 pg/mL。平均MDD为4.67 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FGF-21校正。

E. 线性

不同的样本中含有或掺入高浓度的人FGF-21，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		细胞培养基 (n=4)	人血清 (n=4)	人EDTA血浆 (n=4)	人肝素血浆 (n=4)
1:2	平均值/期待值（%）	105	101	101	102
	范围 (%)	103-107	96-108	99-103	97-105
1:4	平均值/期待值（%）	105	98	102	104
	范围 (%)	102-107	96-103	98-104	99-110
1:8	平均值/期待值（%）	101	98	100	101
	范围 (%)	99-103	94-102	98-101	97-104
1:16	平均值/期待值（%）	95	90	93	101
	范围 (%)	91-104	86-94	90-97	99-105

F. 样本预值

人血清/血浆–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 FGF-21。本研究中使用的供体没有病史。

样本类型	平均检测值 (pg/mL)	%可检测率	范围 (pg/mL)
人血清 (n=35)	172	97	ND-914
人EDTA血浆 (n=35)	201	97	ND-1155
人肝素血浆 (n=35)	186	97	ND-1012

ND=未检测出

细胞培养上清–人外周血白细胞 (PBLs) 在含有5%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。取细胞培养上清液的等分试样，测定人FGF-21的含量。未观察到可检测水平。

G. 特异性

检测方法识别天然和重组人FGF-21。

以下列出的因子在标准品稀释液（1×）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FGF-21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:**
β-ECGF	Flt-3/Flk-2 Ligand	MSPβ	FGF-8b
EGF	Flt-4	MSP R	FGF-8c
EGF (pro)	G-CSF	NGF R	FGF-23
EGF R	G-CSF R	β-NGF	FGF basic
EG-VEGF	GM-CSF	PD-ECGF	FGF R2β
FGF-3	HB-EGF	PDGF-AA	FGF R3
FGF-4	HGF	PDGF-AB	Klotho
FGF-5	HGF Activator	PDGF-BB	**Recombinant rat:**
FGF-6	HGF R	PDGF-CC	FGF basic
FGF-9	HRG-α	PDGF-DD	FGF-BP
FGF-10	IGFBP-1	PDGF Rα	**Recombinant canine:**
FGF-12	IGFBP-2	PDGF Rβ	KGF/FGF-7
FGF-16	IGFBP-3	PlGF	**Natural Proteins:**
FGF-17	IGFBP-4	PPARα	bovine FGF acidic
FGF-18	IGFBP-5	VEGF121	bovine FGF basic
FGF-19	IGFBP-6	VEGF162	human PDGF
FGF-20	IGF-I	VEGF165	porcine PDGF
FGF-22	IGF-I R	VEGF-B167	
FGF-23	IGF-II	VEGF-C (Cys156Ser)	
FGF acidic	IGF-II R	VEGF-C (wild	

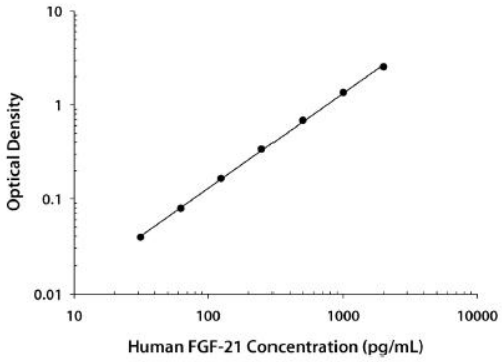
		type)	
FGF basic	IGFBP-rp1	VEGF-D	
FGF R1α	KGF/FGF-7	VEGF/PlGF	
FGF R2α	Klotho	VEGF R1	
FGF R2β	β-Klotho	VEGF R2	
FGF R3	M-CSF	VEGF R3	
FGF R4	M-CSF R		
Flt-3	MSP		

本实验中，重组小鼠 FGF-21在该检测中交叉反应率约为21%。

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)	O.D.	Average	Corrected
0	0.034 0.034	0.034	—
31.3	0.072 0.074	0.073	0.039
62.5	0.111 0.114	0.113	0.079
125	0.198 0.200	0.199	0.165
250	0.361 0.384	0.373	0.339
500	0.708 0.728	0.718	0.684
1000	1.368 1.409	1.389	1.355
2000	2.549 2.594	2.572	2.538

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human FGF-21 Microplate	包被抗人FGF-21抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human FGF-21 Conjugate	酶标检测抗人FGF-21抗体	1瓶
Human FGF-21 Standard	重组人FGF-21标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1S	检测液	1瓶
Calibrator Diluent Concentrate (2×)/ RD6-10	浓缩的标准品稀释液（2×），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℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="4">2-8℃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℃储存，最多30天*</td></tr>
<tr><td>检测液RD1S</td><td>2-8℃储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（2×）/ RD6-10</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，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 ℃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

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。避免反复冻融循环。样本可能需要用标准品稀释液（1×）进行稀释。

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。

B. 检测前准备工作

注意：*使用前请将所有试剂放置于室温。*

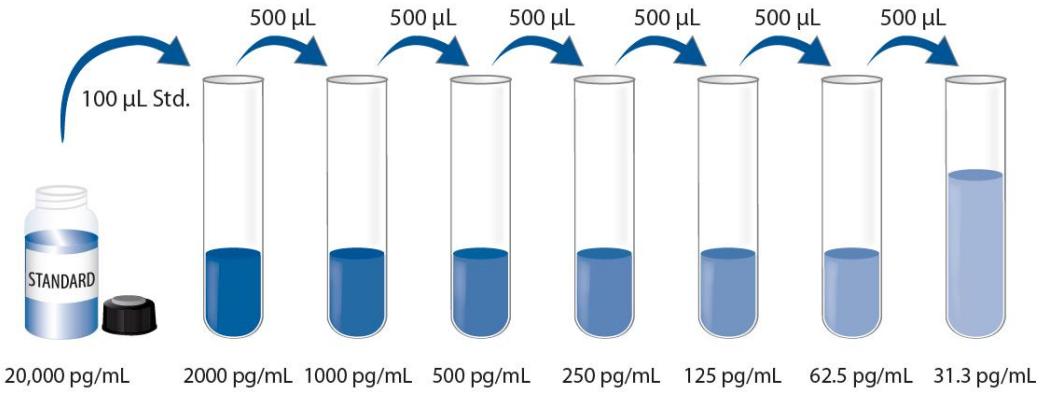
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

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FGF-21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FGF-21标准品，得到浓度为20000 pg/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。

使用聚丙烯管。用移液管将900 µL标准品稀释液（1×）移入2000 pg/mL管子中。用移液管吸取500 µL至其余管子中。使用储备母液溶液稀释（如下）。在下次转移之前，将每根管彻底混合。2000 pg/mL作为最高标准品。标准品稀释液（1×）作为标准品零点（0 pg/mL）。



C. 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。

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100 µL检测液RD1S。
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，**在室温下孵育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200 µL FGF-21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FGF-21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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