PRODUCT INFORMATION & MANUAL

Mouse/Rat Growth and Differentiation Factor-15(GDF-15)

Valukine™ ELISA

Catalog Number: VAL635

For the quantitative determination of natural and recombinant mouse/rat Growth and Differentiation Factor-15(GDF-15) 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

Growth and Differentiation Factor-15 (GDF-15), also known as macrophage inhibitory cytokine-1 (MIC-1), placental transforming growth factor-β, prostate-derived factor, nonsteroidal anti-inflammatory drug-activated gene, and placental bone morphogenetic protein, is a divergent member of the Transforming Growth Factor-β superfamily (1-4). GDF-15 is synthesized as a 40 kDa inactive precursor protein that is proteolytically cleaved to release the active C-terminal fragment, which is then secreted into the circulation as a bioactive disulfide-linked homodimer of 28 kDa.

Under normal conditions, GDF-15 is expressed at high levels in the placenta with lower expression levels in a variety of tissues including the kidney, liver, lung, pancreas, and prostate. It is also expressed in the epithelium of the central nervous system. Its expression by activated macrophages is induced by inflammatory cytokines including IL-1β and TGF-β (1).

GDF-15 has diverse biological functions with roles in inflammation, cancer, and metabolism, and it is associated with all-cause mortality and miscarriage (5-7). GDF-15 is upregulated under inflammatory conditions such as atherosclerosis or rheumatoid arthritis (8-10). Increased expression of GDF-15 is also observed in many cancers (6, 11-14). The metabolic effects of GDF-15 are associated with the modulation of neuronal pathways important in the regulation of appetite and energy homeostasis (15, 16). Increased levels of GDF-15 in the serum of individuals with advanced cancer or chronic disease are correlated with anorexia/cachexia in mice and humans (15, 17).

GDF-15 also exerts cardioprotective actions (18). In mouse models, induction of GDF-15 protects the heart from ischemia/reperfusion injury, and over-expression of GDF-15 attenuates ventricular dilation and heart failure. In humans, serum GDF-15 concentrations are associated with the risk of acute coronary syndrome as well as its prognosis (19, 20).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse/rat GDF-15 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse/rat GDF-15 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse/rat GDF-15 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 mouse/rat GDF-15 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, mouse/rat plasma, mouse/rat serum and mouse/rat 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 twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (pg/mL)	25.6	60.0	190	26.7	63.1	196
Standard Deviation	0.690	1.42	5.49	1.62	2.84	10.7
CV%	2.7	2.4	2.9	6.1	4.5	5.5

B. RECOVERY

The recovery of mouse/rat GDF-15 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range (%)
Cell culture media (n=4)	102	91-110
Serum (n=4)	91	82-101
EDTA plasma (n=4)	95	81-109
Heparin plasma (n=4)	95	87-104
Urine*(n=4)	97	88-106

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

C. SENSITIVITY

Twenty-eight assays were evaluated and the minimum detectable dose (MDD) of mouse/rat GDF-15 ranged from 0.254-2.20 pg/mL. The mean MDD was 0.686 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 mouse GDF-15 produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of mouse/rat GDF-15 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Mouse Samples		**Cell culture supernates* (n=4)**	**Serum (n=4)**	**EDTA plasma (n=4)**	**Heparin plasma (n=4)**	**Urine* (n=4)**
1:2	Average % of Expected	99	102	104	104	99
	Range (%)	95-104	99-104	101-108	103-106	97-101
1:4	Average % of Expected	96	106	105	106	99
	Range (%)	92-99	106-107	99-111	105-108	97-100
1:8	Average % of Expected	96	112	110	110	98
	Range (%)	91-100	107-114	103-118	106-116	95-100
1:16	Average % of Expected	95	117	111	113	97
	Range (%)	89-97	114-121	108-119	108-118	89-102

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

Note: *Rat samples were evaluated and no significant difference in linearity or recovery was observed from the data above.*

F. SAMPLE VALUES

Mouse/Rat serum/plasma - Samples were evaluated for detectable levels of mouse/rat GDF-15 in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
serum (n=10)	77.0	47.4-97.1	19.5
EDTA plasma (n=5)	241	54.4-874	354
Heparin plasma (n=5)	110	82.4-169	34.7
Urine (n=10)	2376	632-6255	1547

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
serum (n=10)	27.5	10.5-49.4	14.3
Heparin plasma (n=5)	42.7	11.1-145	57.3
Urine (n=10)	4972	1922-11550	2747

Rat Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
EDTA plasma (n=5)	31.5	80	ND-68.8

ND=Non-detectable

Cell Culture Supernates:

C2C12 mouse myoblast cells were cultured in DMEM supplemented with 10% fetal bovine serum to 80% confluency. Cell were washed with PBS and cultured in DMEM supplemented with 5% equine serum for 6 days with media changes every other day. When the cells were 50% differentiated, they were unstimulated or stimulated with 1000 µM H_2O_2 for 1 day. Aliquots of the cell culture supernates were removed, assayed for mouse/rat GDF-15, and measured 4630 pg/mL and 7450 pg/mL, respectively.

Hepa 1-6 mouse hepatoma cells (1×10^6 cells/mL) were cultured for 6 days in DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for mouse/rat GDF-15, and measured 6255 pg/mL.

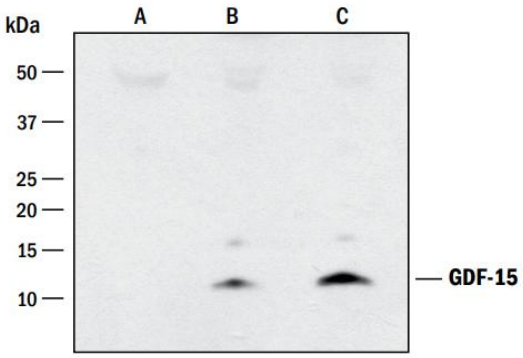
H4-II-E-C3 rat hepatoma cells (2×10^6 cells/mL) were cultured for 3 days in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for mouse/rat GDF-15, and measured 514 pg/mL.

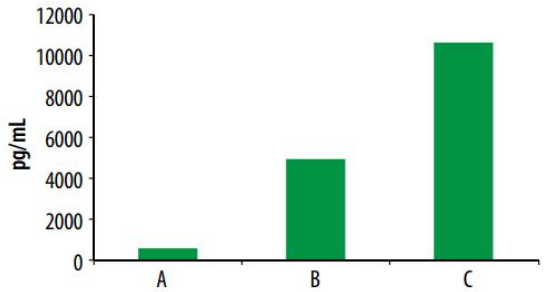
G. SPECIFICITY

This assay recognizes natural mouse and rat GDF-15, recombinant mouse mature GDF-15 and precursor GDF-15. It does not detect monomeric recombinant mouse GDF-15.

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 mouse/rat GDF-15 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:		**Recombinant human:**
GDF-1	GDF-7	GDF-11
GDF-3	GDF-8	GDF-15
GDF-5	GDF-9	
GDF-6	GDF-15 (monomer, aa 189-303)	



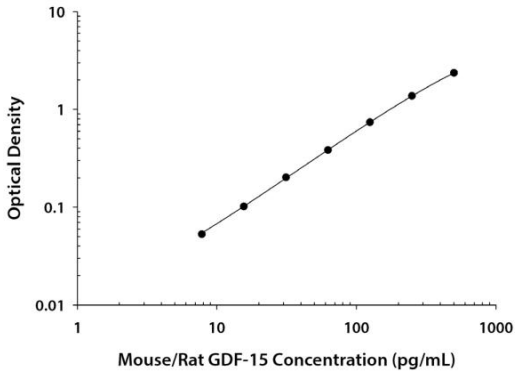


Conditioned media samples from C2C12 mouse myoblast cells were analyzed by Western Blot and Valukine ELISA. (A) The undifferentiated sample was from cells cultured in DMEM supplemented with 10% fetal bovine serum. (B) The differentiated sample was from cells cultured in DMEM supplemented with 5% equine serum for 6 days. (C) Other differentiated media was cultured in DMEM and equine serum and then treated with 1000 µM H_2O_2 for 48 hours. Samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody in this kit. The Western Blot shows a direct correlation with the ELISA value.

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	0.023 0.024	0.024	—
7.8	0.076 0.078	0.077	0.053
15.6	0.124 0.127	0.126	0.102
31.3	0.220 0.231	0.226	0.202
62.5	0.408 0.408	0.408	0.384
125	0.757 0.769	0.763	0.739
250	1.376 1.413	1.395	1.371
500	2.362 2.416	2.389	2.365

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse/Rat GDF-15 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse/rat GDF-15.	1 plate
Mouse/Rat GDF-15 Conjugate	An antibody specific for mouse/rat GDF-15 conjugated to horseradish peroxidase.	1 vial
Mouse/Rat GDF-15 Standard	Recombinant mouse GDF-15 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse/Rat GDF-15 Control	Recombinant mouse GDF-15 in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the label.	2 vials
Assay Diluent RD1W	A buffered protein base.	1 vial
Calibrator Diluent Concentrate (5×)/ RD5P	A 5× 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 vial
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="7">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>Assay Diluent RD1W</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Use a new standard and control for each assay. Discard after use.</td></tr>
<tr><td>Control</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Calibrator Diluent Concentrate (5×)/ RD5P</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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- 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 - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x 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 - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Mouse/Rat urine samples recommend a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 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.

Mouse/Rat GDF-15 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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×).

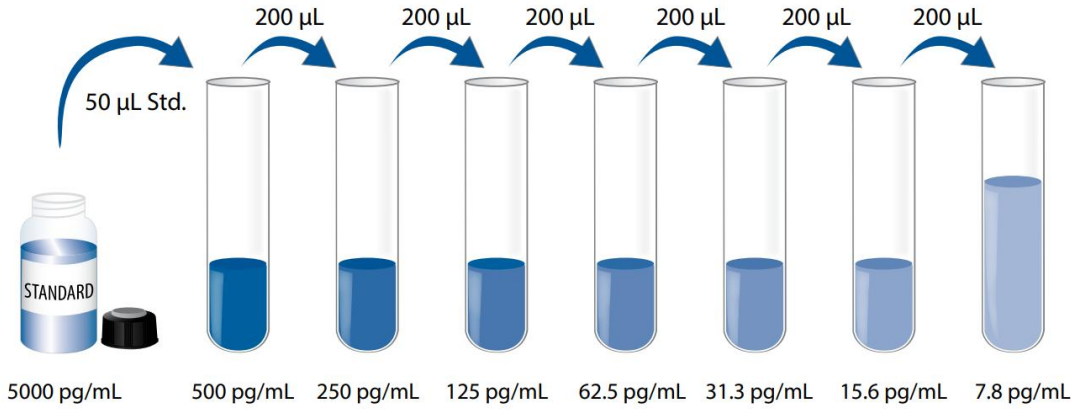
Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Mouse/Rat GDF-15 Standard- Refer to the vial label for the reconstitution

volume* Reconstitute the Mouse/Rat GDF-15 Standard with deionized or distilled water. Do not substitute other diluents. 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.

Pipette 450 µL of Calibrator Diluent (1×) into the 500 pg/mL tube. Pipette 200 µL into each of 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 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, control and standards be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control and samples 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, control and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.** 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 Mouse/Rat GDF-15 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.**
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 on the benchtop. 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, 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 mouse/rat GDF-15 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.

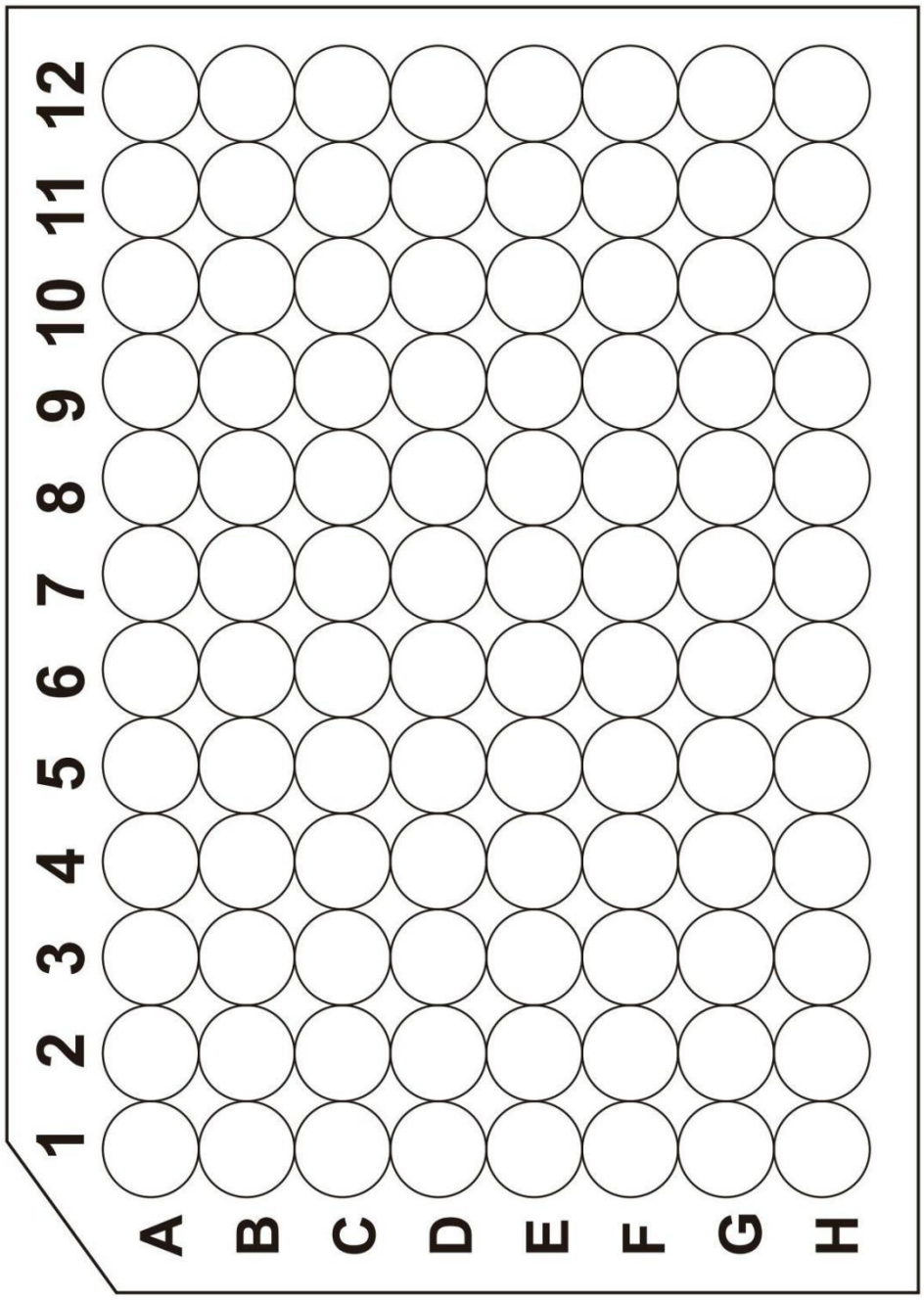
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Bootcov, M.R. et al. (1997) Proc. Natl. Acad. USA 94:11514.
2. Lawton, L.N. et al. (1997) Gene 203:17.
3. Paralkar, V.M. et al. (1998) J. Biol. Chem. 273:13760.
4. Hsiao, E.C. et al. (2000) Mol. Cell. Biol. 20:3742.
5. Wiklund, F.E. et al. (2010) Aging Cell 9:1057.
6. Breit, S.N. et al. (2011) Growth Factors 29:187.
7. Tong, S. et al. (2004) Lancet 363:129.
8. Brown, D.A. et al. (2007) Arthritis Rheum. 56:753.
9. Brown, D.A. et al. (2002) Lancet 359:2159.
10. Taddei, S. and A. Virdis (2010) Eur. Heart J. 31:1168.
11. Vanhara, P. et al. (2012) Prostate Cancer Prostatic Dis. 15:320.
12. Bock, A.J. et al. (2010) Int. J. Gynecol. Cancer 20:1448.
13. Aw Yong, K.M. et al. (2014) J. Cell. Physiol. 229:362.
14. Brown, D.A. et al. (2012) Cancer Epidemiol. Biomarkers Prev. 21:337.
15. Johnen, H. et al. (2007) Nat. Med. 13:1333.
16. Chrysovergis, K. et al. (2014) Int. J. Obes. 38:1555.
17. Tsai, V.W. et al. (2012) J. Cachexia Sarcopenia Muscle 3:239.
18. Ago, T. and J. Sadoshima (2006) Circ. Res. 98:294.
19. Wallentin, L. et al. (2013) PLoS One 8:e78797.
20. Kempf, T. and K.C. Wollert (2009) Herz 34:594.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠/大鼠生长分化因子-15(GDF-15) Valukine™ ELISA 试剂盒

目录号：VAL635

适用于定量检测天然和重组小鼠/大鼠生长分化因子-15（GDF-15）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

GDF-15 又称 MIC-1、胎盘转化生长因子-β、前列腺衍生因子、非甾体类抗炎药物激活基因和胎盘骨形态发生蛋白，它是 TGF-β超家族中的一个分化成员（1-4）。GDF-15 合成为 40 kDa 的非活性前体蛋白，经蛋白水解后释放出活性 C 端片段，然后以具有生物活性分子量为 28 kDa 二硫键组成的同源二聚体的形式分泌到血液循环中。

正常情况下，GDF-15 在胎盘中的表达水平较高，而在肾脏、肝脏、肺、胰腺和前列腺等多种组织中的表达水平较低。它还在中枢神经系统的上皮细胞中表达。包括 IL-1β 和 TGF-β 在内的炎症细胞因子会诱导活化的巨噬细胞表达该蛋白 (1)。

GDF-15 具有多种生物功能，在炎症、癌症和新陈代谢中发挥作用，并且与全因死亡率和流产有关（5-7）。GDF-15 在动脉粥样硬化或类风湿性关节炎等炎症条件下会上调（8-10）。在许多癌症中也观察到 GDF-15 的表达增加（6, 11-14）。GDF-15 的代谢作用与调节食欲和能量平衡的神经元通路有关（15, 16）。在小鼠和人类的晚期癌症或慢性病患者血清中 GDF-15 水平的升高与厌食-恶病质综合症有关（15, 17）。

GDF-15 还具有保护心脏的作用（18）。在小鼠模型中，诱导 GDF-15 可保护心脏免受缺血/再灌注损伤，过度表达 GDF-15 可减轻心室扩张和心力衰竭。在人体中，血清中 GDF-15 的浓度与急性冠状动脉综合征的风险及其预后有关（19, 20）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠/大鼠GDF-15抗体包被于微孔板上，样品，质控品和标准品中的小鼠/大鼠GDF-15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小鼠/大鼠GDF-15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，小鼠/大鼠血浆样本，小鼠/大鼠血清样本和小鼠/大鼠尿液样本；
- 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20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	25.6	60.0	190	26.7	63.1	196
标准差	0.690	1.42	5.49	1.62	2.84	10.7
CV%	2.7	2.4	2.9	6.1	4.5	5.5

B. 回收率

在不同类型样本中掺入检测范围内不同水平的小鼠/大鼠GDF-15，测定其回收率。

小鼠样本	平均回收率%	范围（%）
细胞培养基（n= 4）	102	91-110
血清（n=4）	91	82-101
EDTA血浆（n=4）	95	81-109
肝素血浆（n=4）	95	87-104
尿液*（n=4）	97	88-106

*样品在检测前按照样品制备部分的指示进行样本稀释。

C. 灵敏度

28次检测结果表明，小鼠/大鼠的GDF-15最低检测剂量（MDD）的范围为 0.254-2.20 pg/mL。平均MDD为0.686 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小鼠GDF-15校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠/大鼠GDF-15，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。

小鼠样本		细胞培养上清（n=4）	血清（n=4）	EDTA血浆（n=4）	肝素血浆（n=4）	尿液*（n=4）
1:2	平均值/期待值（%）	99	102	104	104	99
	范围（%）	95-104	99-104	101-108	103-106	97-101
1:4	平均值/期待值（%）	96	106	105	106	99
	范围（%）	92-99	106-107	99-111	105-108	97-100
1:8	平均值/期待值（%）	96	112	110	110	98
	范围（%）	91-100	107-114	103-118	106-116	95-100
1:16	平均值/期待值（%）	95	117	111	113	97
	范围（%）	89-97	114-121	108-119	108-118	89-102

*样品在测定前按照样品制备部分的指示进行稀释。

注意：*对大鼠样本进行评估，上述数据的线性和回收率没有显著差异。*

F. 样本预值

小鼠/大鼠血清/血浆样本 - 在此测定中对样品进行小鼠/大鼠GDF-15的可检测水平的评估。

小鼠样本	平均值(pg/mL)	范围 (pg/mL)	标准偏差 (pg/mL)
血清 (n=10)	77.0	47.4-97.1	19.5
EDTA血浆 (n=5)	241	54.4-874	354
肝素血浆 (n=5)	110	82.4-169	34.7
尿液 (n=10)	2376	632-6255	1547

大鼠样本	平均值 (pg/mL)	范围(pg/mL)	标准偏差(pg/mL)
血清 (n=10)	27.5	10.5-49.4	14.3
肝素血浆 (n=5)	42.7	11.1-145	57.3
尿液 (n=10)	4972	1922-11550	2747

大鼠样本	可检测平均值 (pg/mL)	可检测率%	范围 (pg/mL)
EDTA血浆 (n=5)	31.5	80	ND-68.8

ND = 未检出

细胞上清样本 - C2C12 小鼠成肌细胞培养在含10%胎牛血清的DMEM培养基中，培养至 80% 汇合度。用PBS冲洗细胞，然后再培养在含5%马血清的DMEM培养基中，培养6天，每隔一天更换一次培养基。当细胞分化到50%时，用1000 μM H_2O_2刺激细胞1天。取出等量的细胞培养上清，检测小鼠/大鼠GDF-15，检测值分别为 4630 pg/mL和7450 pg/mL。

Hepa 1-6 小鼠肝癌细胞（1 × 10^6 cells/mL）培养在含10%胎牛血清和2 mM L-谷氨酰胺的 DMEM 培养基中，培养6天。取出等量的细胞培养上清，检测小鼠/大鼠GDF-15，检测值为 6255 pg/mL。

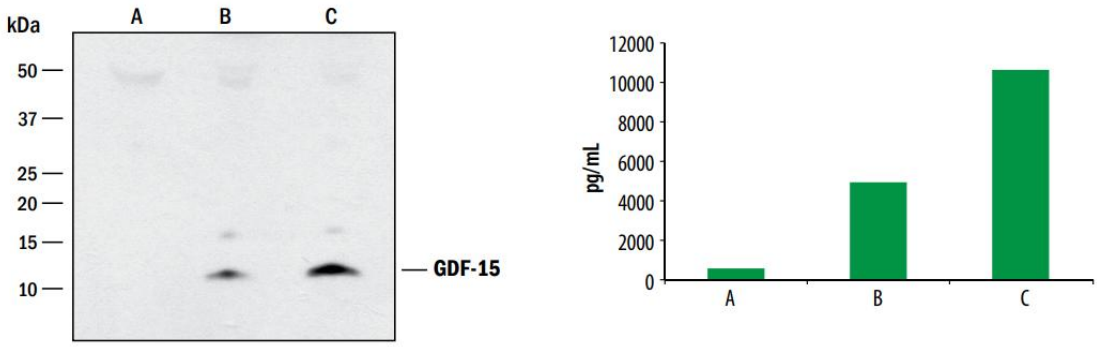
H4-II-E-C3 大鼠肝癌细胞（2 × 10^6 cells/mL）培养在含10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL 硫酸链霉素的 DMEM培养基中，培养3天。取出等量的细胞培养上清，检测小鼠/大鼠GDF-15，检测值为514 pg/mL。

G. 特异性

此ELISA法可检测天然小鼠及大鼠GDF-15、重组小鼠成熟GDF-15和前体GDF-15。不能检测单体重组小鼠GDF-15。

将以下表用标准品稀释液（1×）配制成50 ng/mL的浓度来检测与小鼠GDF-15的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠/大鼠GDF-15对照品中，来检测对小鼠/大鼠GDF-15的干扰。未观察到明显的交叉反应或干扰

Recombinant mouse:		**Recombinant human:**
GDF-1	GDF-7	GDF-11
GDF-3	GDF-8	GDF-15
GDF-5	GDF-9	
GDF-6	GDF-15 (monomer, aa 189-303)	

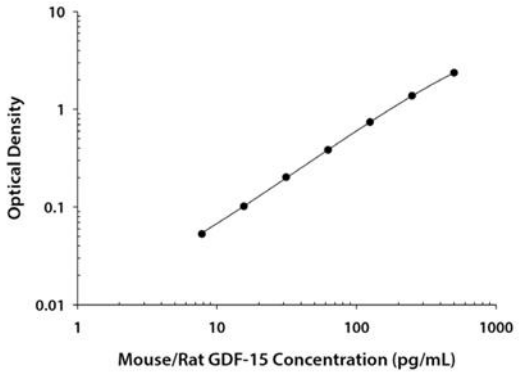


通过 Western Blot 和 Valukine ELISA分析C2C12小鼠成肌细胞的条件培养基样本。（A）未分化样本来自用添加 10%胎牛血清的DMEM 培养的细胞。（B）分化样本来自用添加 5%马血清的DMEM培养6天的细胞。（C）其他分化培养基在DMEM和马血清中培养，然后用1000 μM H_2O_2处理48小时。在还原SDS-PAGE条件下分辨样品，转移到PVDF膜上，用本试剂盒中的检测抗体进行免疫印迹。Western印迹结果与ELISA值直接相关。

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	0.023 0.024	0.024	—
7.8	0.076 0.078	0.077	0.053
15.6	0.124 0.127	0.126	0.102
31.3	0.220 0.231	0.226	0.202
62.5	0.408 0.408	0.408	0.384
125	0.757 0.769	0.763	0.739
250	1.376 1.413	1.395	1.371
500	2.362 2.416	2.389	2.365

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse/Rat GDF-15 Microplate	包被抗小鼠/大鼠GDF-15抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse/Rat GDF-15 Conjugate	酶标检测抗小鼠/大鼠GDF-15抗体	1瓶
Mouse/Rat GDF-15 Standard	小鼠GDF-15标准品（冻干），参考瓶身标签进行重溶	2瓶
Mouse/Rat GDF-15 Control	小鼠GDF-15质控品（冻干），质控品的测定值应在标签上规定的范围内	2瓶
Assay Diluent RD1W	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5P	浓缩标准品稀释液（5×）用于稀释标准品和样本	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="5">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1W</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>质控品</td><td rowspan="2">每次检测均使用新的标准及对照，用后丢弃</td></tr>
<tr><td>标准品</td></tr>
<tr><td>浓缩标准品稀释液(5×)/RD5P</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450nm检测波长的吸收值及540nm或570nm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
- 100mL和500mL量筒
- 水平振荡器（0.12” 轨道），转速：500±50 rpm
- 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：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

血清样本：血液样品在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20℃储存备用。避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

血浆样本：使用EDTA或肝素作为抗凝剂收集血浆。然后2000 × g离心20分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤ -20℃储存备用。避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

注意：*本试剂盒对柠檬酸钠血浆尚未被验证。*

尿液：使用代谢笼收集尿液。离心去除微粒并立即化验，或将样品等分并保存在 ≤ -20℃的温度下。避免反复冻融循环。化验前再次离心，去除储存后可能出现的沉淀物。样本可能需要用标准品稀释液（1×）稀释。

B. 样本准备工作

小鼠/大鼠尿液样本建议用标准品稀释液（1×）50倍稀释后进行检测，即10 µL样品+490 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温。

小鼠/大鼠GDF-15质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

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×）。

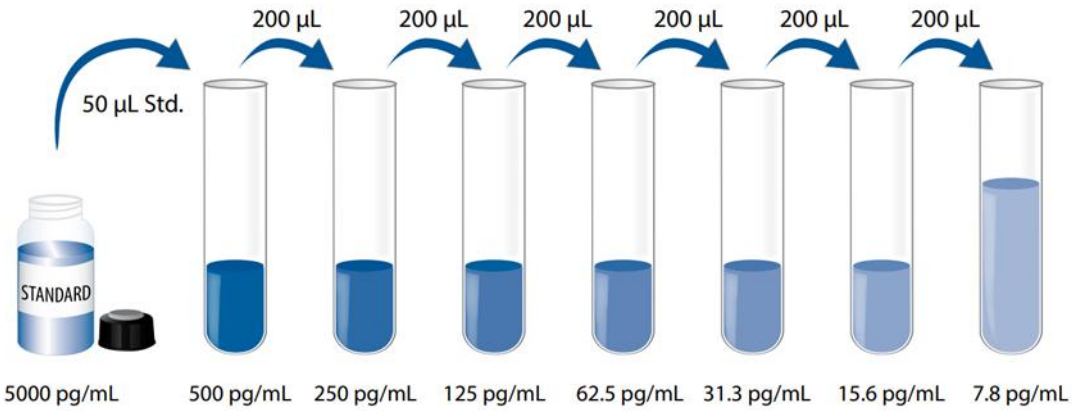
标准品稀释液（1×）：使用蒸馏水或去离子水稀释配制成标准品稀释液（1×）。

小鼠/大鼠GDF-15标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重溶小鼠/大鼠GDF-15 标准品。不得使用其他试剂。得到浓度为5000 pg/mL标准品母液。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。

加入450 µL标准品稀释液（1×）到 500 pg/mL管中。剩余每管中加入200 µL标准品稀

释液（1×）。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。500 pg/mL作标准曲线最高点，标准品稀释液（1×）可用作标准曲线零点（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，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，质控品和标准品做复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂，标准品，质控品和样本；
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. 每孔加入50μL检测液RD1W。
4. 分别将不同浓度标准品，质控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，**在水平振荡器（0.12”轨道）转速：500±50 rpm上，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小鼠/大鼠GDF-15酶标检测抗体。用封板膜封住反应孔，**在水平振荡器（0.12”轨道）转速：500±50 rpm上，室温孵育2小时；**重复第5步洗板操作；
7. 重复第五部洗板操作；
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，质控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轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制小鼠/大鼠GDF-15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. Bootcov, M.R. et al. (1997) Proc. Natl. Acad. USA 94:11514.
2. Lawton, L.N. et al. (1997) Gene 203:17.
3. Paralkar, V.M. et al. (1998) J. Biol. Chem. 273:13760.
4. Hsiao, E.C. et al. (2000) Mol. Cell. Biol. 20:3742.
5. Wiklund, F.E. et al. (2010) Aging Cell 9:1057.
6. Breit, S.N. et al. (2011) Growth Factors 29:187.
7. Tong, S. et al. (2004) Lancet 363:129.
8. Brown, D.A. et al. (2007) Arthritis Rheum. 56:753.
9. Brown, D.A. et al. (2002) Lancet 359:2159.
10. Taddei, S. and A. Virdis (2010) Eur. Heart J. 31:1168.
11. Vanhara, P. et al. (2012) Prostate Cancer Prostatic Dis. 15:320.
12. Bock, A.J. et al. (2010) Int. J. Gynecol. Cancer 20:1448.
13. Aw Yong, K.M. et al. (2014) J. Cell. Physiol. 229:362.
14. Brown, D.A. et al. (2012) Cancer Epidemiol. Biomarkers Prev. 21:337.
15. Johnen, H. et al. (2007) Nat. Med. 13:1333.
16. Chrysovergis, K. et al. (2014) Int. J. Obes. 38:1555.
17. Tsai, V.W. et al. (2012) J. Cachexia Sarcopenia Muscle 3:239.
18. Ago, T. and J. Sadoshima (2006) Circ. Res. 98:294.
19. Wallentin, L. et al. (2013) PLoS One 8:e78797.
20. Kempf, T. and K.C. Wollert (2009) Herz 34:594.

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