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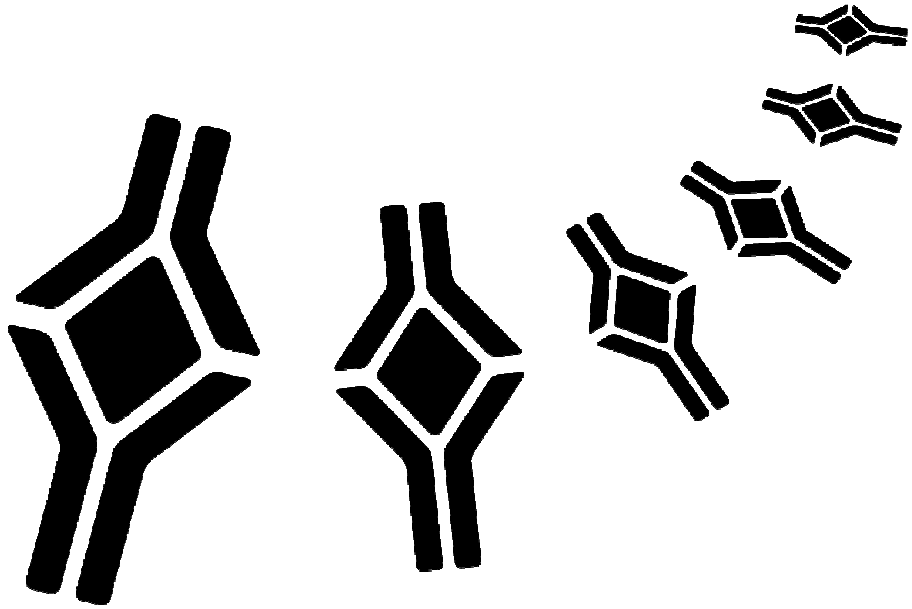
Research
and Diagnostic Products



HUMAN GDF-15/MIC-1 ELISA

Product Data Sheet

Cat. No.: RD191135200R

For Research Use Only

VERSION 99 160311 19

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD191135200R Human GDF-15 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human GDF-15/MIC-1 (growth differentiation factor 15 / macrophage-inhibitory cytokine 1).

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures human GDF-15 in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Growth differentiation factor 15 (**GDF-15**) is a member of the transforming growth factor β (TGF- β) cytokine superfamily. GDF-15 was originally cloned as macrophage-inhibitory cytokine 1 (**MIC-1**) and later also identified as placental TGF–β, placental bone morphogenetic protein (PLAB), nonsteroidal antiinflammatory drug-activated gene 1, and prostate-derived factor. Like other TGF- β–related cytokines, GDF-15 is synthesized as a 62-kDa precursor protein, which, after cleavage by furin-like protease, is secreted as 25-kDa disulfide-linked dimmer.

GDF-15 is produced in low amounts under baseline conditions in most tissues such as brain, liver, kidney, pancreas, but not normally in many other organs including the heart. It is highly expressed in placenta and moderately in prostate.

GDF-15 expression levels increased markedly in the heart in mouse models of myocardial infarction and in heart disease. Cell culture experiments indicate that GDF-15 is involved in the execution of cell death programs in neurons and tumor cell lines. More recent studies of GDF-15 gene-targeted mice indicate that GDF-15 functions as a cardioprotective cytokine during myocardial infarction and heart failure. These studies along with cell culture studies using recombinant GDF-15 demonstrate a functional role for GDF-15 in vivo.

GDF-15 is also upregulated by other cardiovascular events triggering oxidative stress, including pressure overload, and atherosclerosis.

Moreover, increased circulating GDF-15 concentrations have been linked to an enhanced risk of future adverse cardiovascular events in elderly women and it was describe as a new biomarker of the risk of death in patients with non-ST-elevation acute coronary syndrome.

Serum GDF-15 concentrations increase in maternal serum with advancing gestation in normal pregnancy. Low GDF-15 concentrations reportedly are associated with an increased risk of preterm labor or miscarriage.

Increased GDF-15 expression has been documented in a variety of epithelial cell lines, including breast, pancreas, colorectal, and prostate cancers. Microarray studies have revealed increased expression of GDF-15 in patients with breast cancer, and serum GDF-15 levels are the best single predictor of the presence of pancreatic carcinoma. In the case of prostate cancer, serum GDF-15 levels increase with progression of disease to metastasis. In colon cancer, increasing GDF-15 expression is associated with the progression of colonic adenomas to invasive cancer and subsequent metastasis, with serum levels at presentation being an independent predictor of subsequent disease-free status and overall survival.

GDF-15 levels in blood plasma have been found to be dramatically elevated in beta-thalassemia patients compared to healthy donors and patients with hereditary hemochromatosis, another from of iron overload disease. In addition, the role of GDF-15 in other disorders characterized by ineffective erythropoiesis, as well as the role of GDF-15 in regulation of iron metabolism is under investigation. There are some hypotheses for treatment of thalassemia by administration of GDF-15 antagonist, and to reduce hepcidin levels by administration of GDF-15, a GDF-15 substitute, or GDF-15 agonist.

Areas of investigation:
Cardiology
Pregnancy
Oncology
Hematology

4. TEST PRINCIPLE

In the BioVendor Human GDF-15/MIC-1 ELISA, the standards, quality controls and samples are incubated in microtitrate wells pre-coated with polyclonal anti-human GDF-15 antibody. After 60 min incubation and a washing, biotin labelled polyclonal anti-human GDF-15 antibody is added and incubated with captured GDF-15 for 60 min. After another washing, the streptavidin-HRP conjugate is added. After 30 min incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of GDF-15. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (50x)	concentrate	0.26 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Biotin Ab - Diluent	ready to use	13 ml
Dilution Buffer	ready to use	20 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Instruction Manual + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1 000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

» **All reagents need to be brought to room temperature prior to use**

» **Always prepare only the appropriate quantity of reagents for your test**

» **Do not use components after the expiration date marked on their label**

- **Assay reagents supplied ready to use:**

Antibody Coated Microtiter Strips
Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Biotin Ab-Diluent
Streptavidin-HRP Conjugate
Substrate Solution
Stop Solution
Dilution Buffer
Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

- **Assay reagents supplied concentrated or lyophilized:**

Human GDF-15 Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human GDF-15 in the stock solution is **8 960 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
stock	-	8 960 pg/ml
250 µl of stock	250 µl	4 480 pg/ml
250 µl of 4 480 pg/ml	250 µl	2 240 pg/ml
250 µl of 2 240 pg/ml	250 µl	1 120 pg/ml
250 µl of 1 120 pg/ml	250 µl	560 pg/ml
250 µl of 560 pg/ml	250 µl	280 pg/ml
250 µl of 280 pg/ml	250 µl	140 pg/ml
250 µl of 140 pg/ml	250 µl	70 pg/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Reconstituted Master Standard must be used immediately or aliquoted and stored frozen at –20 °C for 3 months. Avoid repeating freezing/thawing cycles.
Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
The reconstituted Quality Controls are ready to use, do not dilute them.
Stability and storage:
The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted Quality Controls.

Biotin Labelled Antibody Conc. (50x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (50x) with 49 parts Biotin-Ab Diluent. Example: 20 µl of Biotin Labelled Antibody Concentrate (50x) + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Opened Biotin Labelled Antibody Concentrate (50x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.
Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures GDF-15 in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze-thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Preparation of serum and plasma samples:

Dilute serum or plasma samples 5x with Dilution Buffer just prior to the assay, e.g. 30 µl of sample + 120 µl of Dilution Buffer when assaying samples as singlets or preferably 60 µl of sample + 240 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Preparation of serum and plasma samples from pregnant women:

Dilute serum or plasma samples 15x with Dilution Buffer just prior to the assay, e.g. 10 µl of sample + 140 µl of Dilution Buffer when assaying samples as singlets or preferably 20 µl of sample + 280 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of GDF-15.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards, reconstituted Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Pipet **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
11. Incubate the plate for **10 minutes** at room temperature. (The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C). No shaking!
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550-650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine GDF-15 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 4 480**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 2 240**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 1 120**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 560**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 280**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 140**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 70**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the absorbance (Y) of Standards against *log* of the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of GDF-15 (pg/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of standards).

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay; e.g. 362.3 pg/ml (from standard curve) x 5 (dilution factor) = 1 811.5 pg/ml.

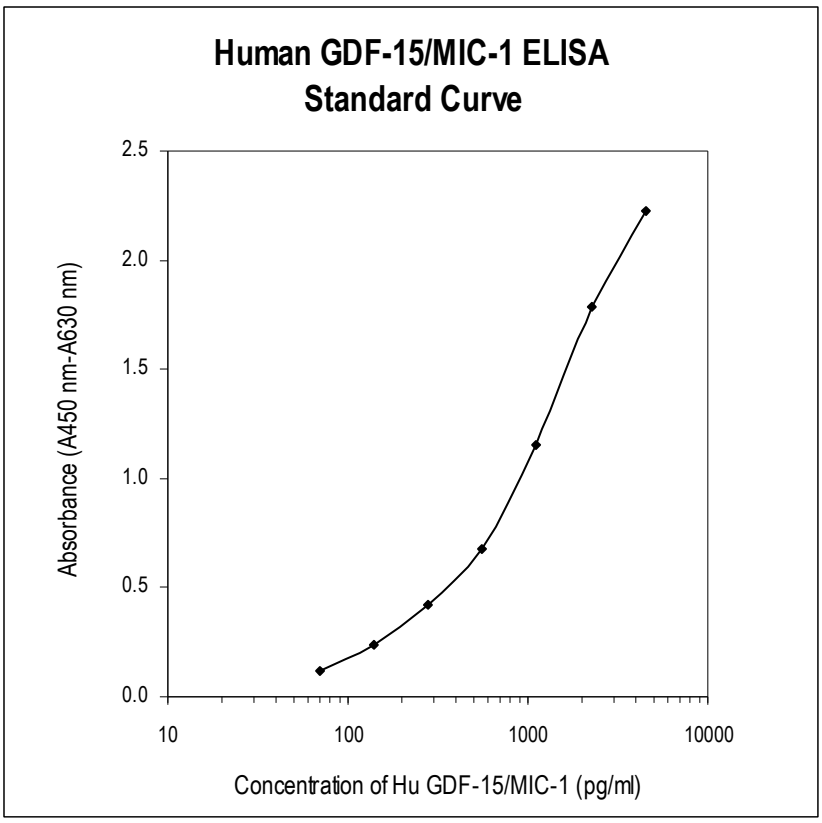


Figure 2: Typical Standard Curve for Human GDF-15/MIC-1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human GDF-15/MIC-1 ELISA are presented in this chapter

- **Sensitivity**

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real GDF-15 values in wells and is 22 pg/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding serum/plasma GDF-15 level of 4 480 pg/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the GDF-15 concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human GDF-15.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

» **Presented results are multiplied by respective dilution factor**

- **Precision**

Intra-assay (Within-Run) (n=8)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
1	7 279.7	456.9	6.3
2	870.9	62.9	7.2

Inter-assay (Run-to-Run) (n=8)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
1	1 771.0	150.6	8.5
2	397.8	37.6	9.5

- **Spiking Recovery**

Serum samples were spiked with different amounts of human GDF-15 and assayed.

Sample	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
1	1 650.2	-	-
	4 520.9	4 450.2	101.6
	3 070.6	3 050.2	100.7
	2 136.9	2 350.2	90.9
2	1 051.95	-	-
	3 666.6	3 851.9	95.2
	2 316.7	2 451.9	94.5
	1 661.6	1 751.9	94.8

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
1	-	8 719.2	-	-
	2x	4 053.5	4 359.6	93.0
	4x	1 949.1	2 179.8	89.4
	8x	873.1	1 089.9	80.1
2	-	7 694.1	-	-
	2x	3 888.9	3 847.0	101.1
	4x	1 897.7	1 923.5	98.7
	8x	943.0	961.8	98.0

- **Effect of sample matrix**

Heparin, citrate and EDTA plasmas were compared to respective serum samples from the same 10 individuals.
Results are shown below:

Volunteer No.	*Serum (pg/ml)*	*Plasma (pg/ml)*		
		Heparin	*Citrate*	*EDTA*
1	3 259.0	3 449.2	2 709.7	3 420.6
2	1 301.9	1 473.9	1 173.9	1 404.9
3	1 482.5	1 233.6	1 038.1	1 379.2
4	3 942.8	4 299.4	3 767.1	5 044.5
5	1 856.8	1 830.5	1 190.9	1 586.3
6	1 577.6	1 594.9	1 491.1	2 033.3
7	978.9	902.9	768.3	919.8
8	2 599.8	2 682.1	2 517.9	3 249.8
9	7 561.5	8 130.1	6 006.5	7 873.9
10	2 884.9	3 005.7	2 463.4	2 949.8
Mean (pg/ml)	**2 744.6**	**2 990.2**	**2 312.7**	**2 860.2**
Mean Plasma/Serum (%)	**-**	**109.0**	**84.3**	**104.2**
Coefficient of determination R^2	**-**	**0.99**	**0.98**	**0.98**

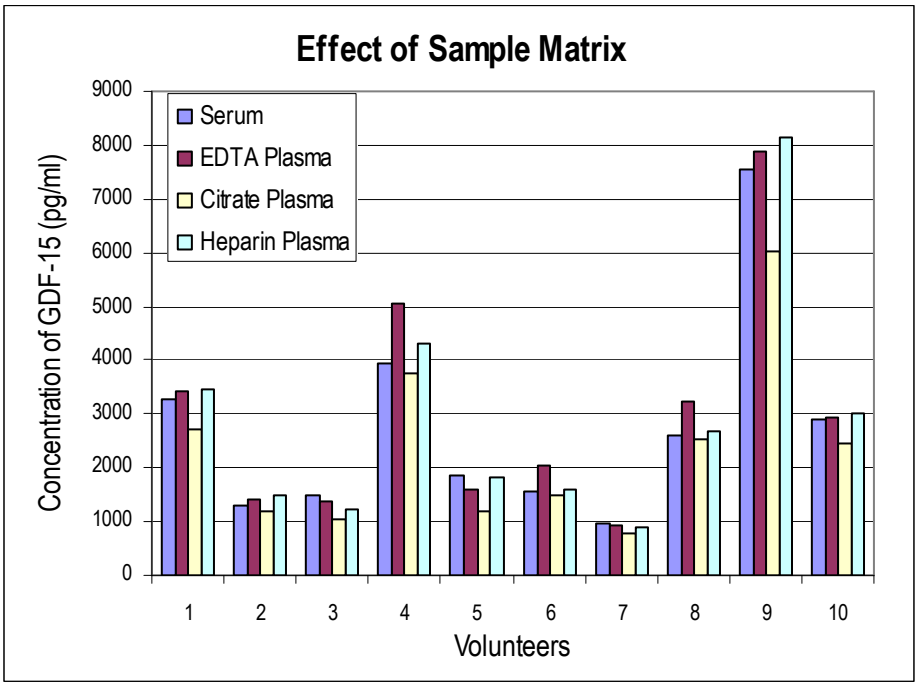


Figure 3: GDF-15 levels measured using Human GDF-15/MIC-1 ELISA from 10 individuals using serum, heparin, citrate and EDTA plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at –20°C. However, no decline in concentration of GDF-15 was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.05%, respectively.

Sample	*Incubation Temp, Period*	*Serum (pg/ml)*	*Plasma (pg/ml)*		
			Heparin	*Citrate*	*EDTA*
1	-20°C	582.2	696.5	514.7	525.5
	2-8°C, 1 day	643.7	661.3	529.2	575.8
	2-8°C, 7 days	502.6	467.4	557.9	661.3
2	-20°C	977.6	946.6	763.2	936.3
	2-8°C, 1 day	922.6	818.8	787.5	874.1
	2-8°C, 7 days	891.5	956.9	790.8	643.5
3	-20°C	525.5	738.7	547.2	564.7
	2-8°C, 1 day	682.3	661.2	618.8	482.0
	2-8°C, 7 days	593.3	639.3	929.5	714.2

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human GDF-15 in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	*Number of f/t cycles*	*Serum (pg/ml)*	*Plasma (pg/ml)*		
			Heparin	*Citrate*	*EDTA*
1	1x	359.7	466.2	382.3	371.7
	3x	395.6	489.9	385.6	448.5
	5x	415.5	445.8	389.0	406.2
2	1x	523.5	618.6	474.8	595.4
	3x	469.5	568.3	406.9	437.2
	5x	606.0	593.4	545.9	593.4
3	1x	1 212.0	1 332.7	990.8	1 118.0
	3x	1 063.3	1 264.8	849.5	1 110.9
	5x	1 135.7	1 042.0	1 086.6	1 148.6

14. DEFINITION OF THE STANDARD

In this assay, the recombinant protein is used as a Standard. The protein was expressed as a fused protein in Chinese Hamster cell line, CHO. The recombinant mature human GDF-15, generated by proteolytic removal of the CD33 signal peptide region, is a disulfide-linked homodimer. Calculated molecular weight is 13 kDa.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum from 142 unselected donors (87 women + 55 men), 6-86 years old were assayed with Biovendor Human GDF-15/MIC-1 ELISA kit in our laboratory.

- **Age and Sex - Dependent Distribution of GDF-15 Values**

Sex	*Age (years)*	*n*	*Mean*	*SD*	*Min.*	*Max.*	*Median*
			GDF-15 (pg/ml)				
Men	14 - 19	2	573.5	20.3	553.2	593.7	573.5
	21 - 49	20	840.8	272.9	386.6	1 344.3	814.9
	50 - 85	33	2 972.5	2 437.0	758.0	11 340.0	2 162.0
Women	6 - 18	6	852.1	251.0	581.3	1 204.7	743.3
	20 - 49	32	980.0	586.2	402.8	3 307.8	798.9
	50 - 86	49	2 584.4	2 067.5	658.5	13 652.8	1 986.5

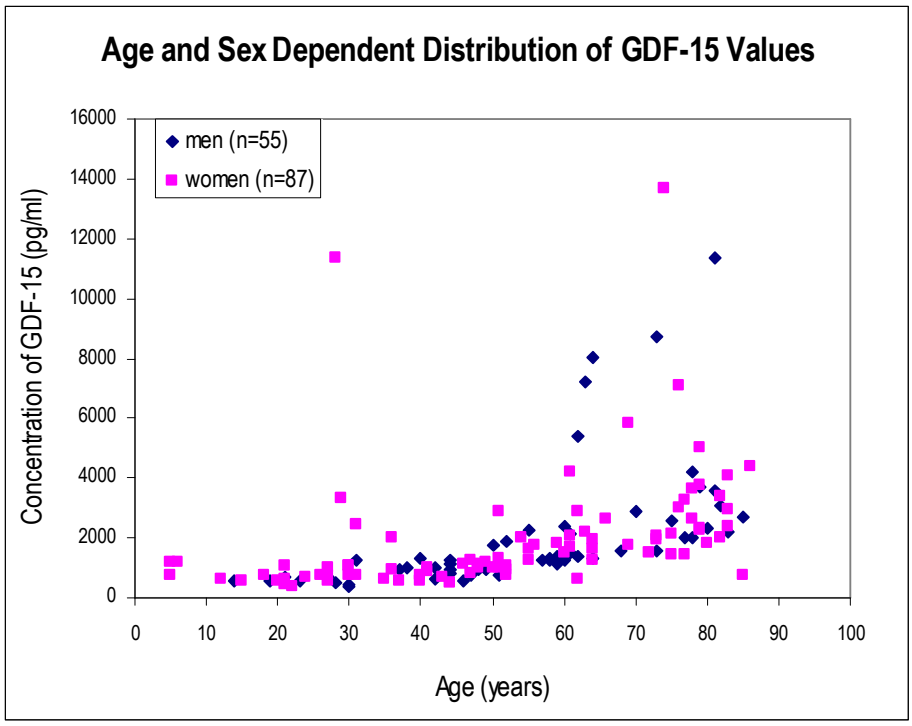


Figure 4: GDF-15 concentration plotted against donor age.

- **Reference range**

It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for GDF-15 levels with the assay.

16. METHOD COMPARISON

Human GDF-15/MIC-1 ELISA has not been compared to any other immunoassay.

17. TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

18. REFERENCES

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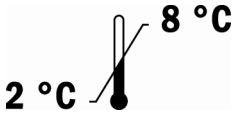
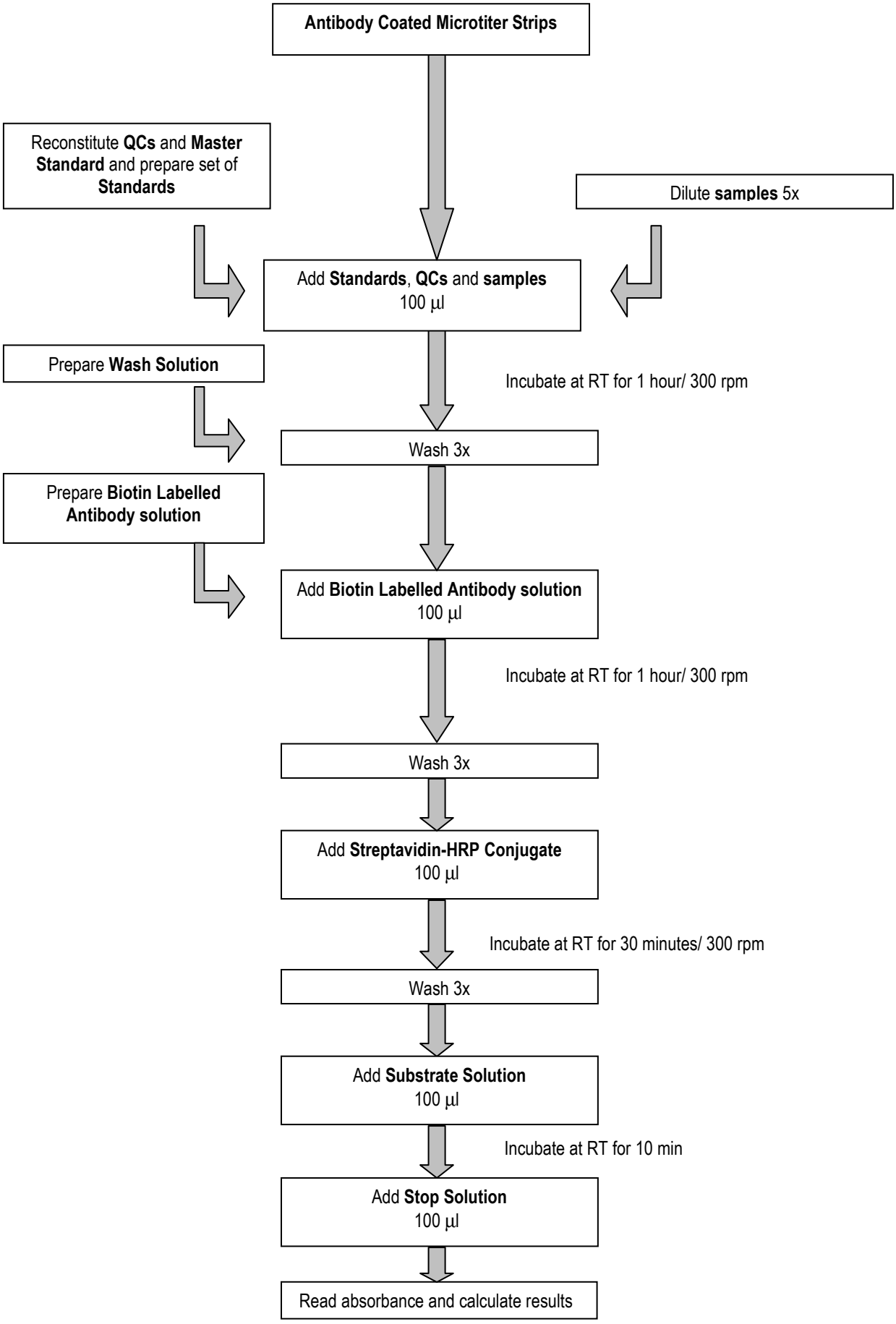
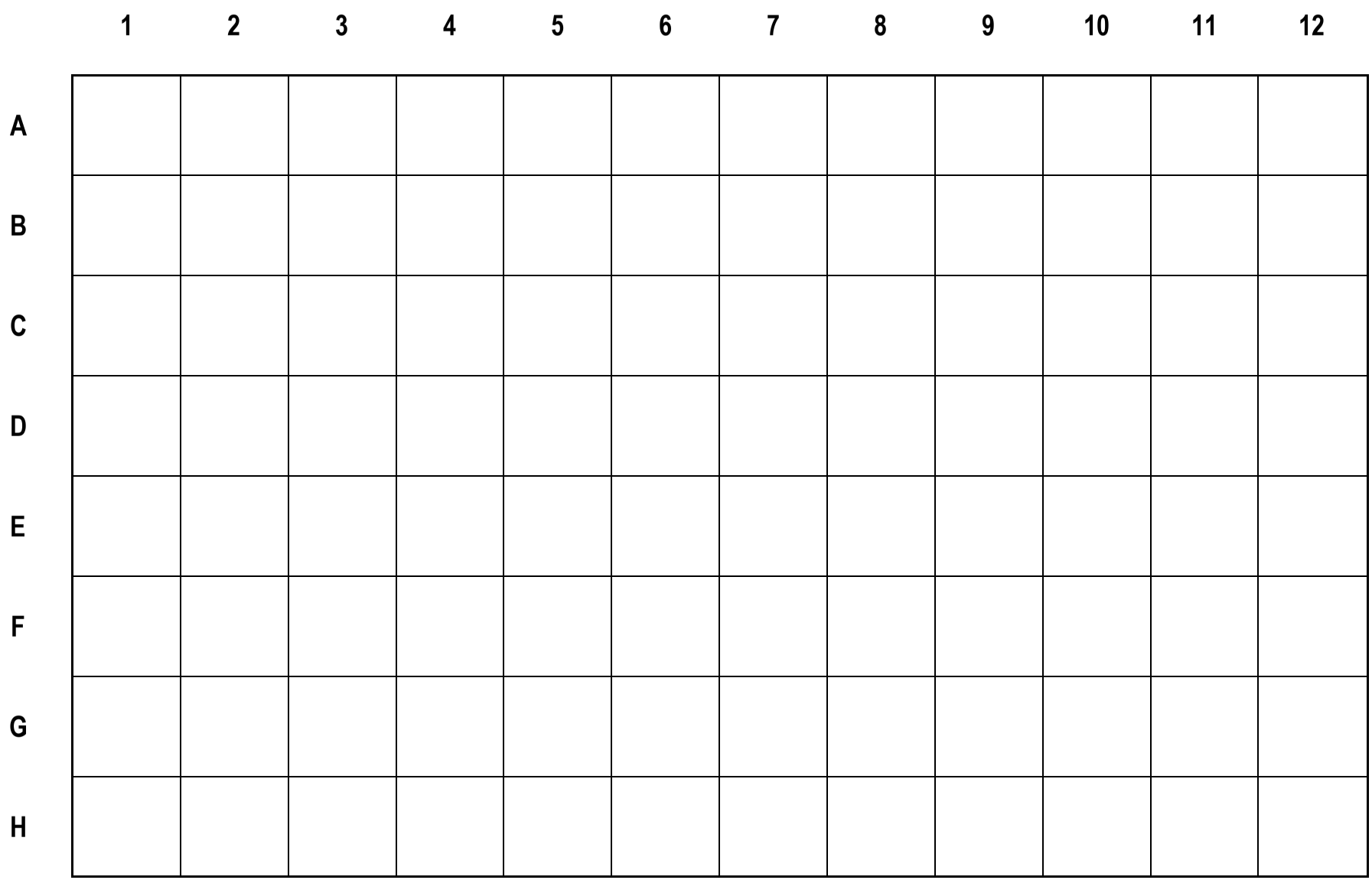
» For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

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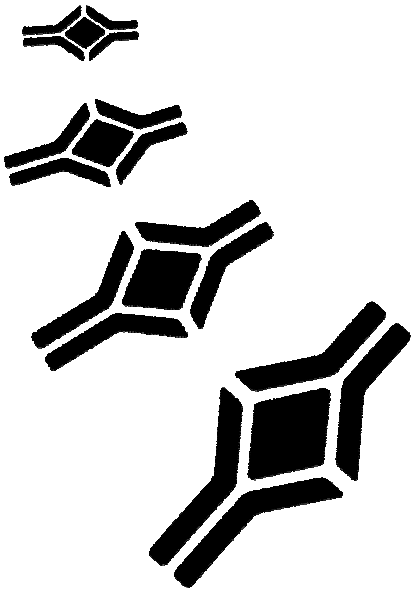

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