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HUMAN GROWTH DIFFERENTIATION FACTOR 15 ELISA

Cat. No.: PL1025

Enzyme Immunoassay for the quantitative determination of Growth differentiation factor 15 (GDF15) in human serum and plasma.

Growth differentiation factor 15 (GDF15) is a member of the TGFß superfamily whose expression is increased in response to cellular stress and disease as well as by metformin.1 Identified as a new heart-derived endocrine hormone that regulates body growth, GDF15 has a local cardioprotective role, presumably due to its autocrine/paracrine properties: antioxidative, anti-inflammatory, antiapoptotic. GDF15 expression is highly induced in cardiomyocytes after ischemia/reperfusion and in the heart within hours after myocardial infarction (MI). GDF15 may be a predictive biomarker of adverse cardiac events.[2] Available evidence also suggests that a substantial amount of GDF15 is secreted in various human cancers, such as ovarian cancer, prostate cancer, and breast cancer, among others.[3]

Principle of of GDF15 ELISA

The microtiter plate is coated with the antibody specifically binding the Growth differentiation factor 15. The human serum or plasma is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of GDF15 in the specimen. The concentration of GDF15 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

Add 100 µL of Standards, diluted QCs and Samples to the wells

Incubate for 1 hour at 25 °C, shaking at 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of HRP-conjugated Antibody to the wells

Incubate for 1 hour at 25 °C, shaking at 300 rpm

3-times wash the wells (350 µL/well)

Add 100 µL of Substrate Solution to the wells

Incubate for 20 min in the dark at 25 °C, NO shaking

Add 100 µL of Stop Solution to the wells

Read the signal at 450 nm (450/630 nm) within 15 min

Kit contents

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Antibody-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	2×13 mL
Wash Buffer 15× conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

Material required but not supplied

1. Glassware and test tubes.
2. Microtiter plate washer.
3. Precision pipettes (various volumes) with tips.
4. Orbital shaker.
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation.

Warnings and precautions

1. For research use only.
2. For professional laboratory use.
3. The reagents with different lot numbers should not be mixed.
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25 °C ±2 °C).

Storage conditions

1. The kit must be stored at 2–8 °C.
2. The opened components can be stored for one week at 2–8 °C.

Preparation of reagents

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25 °C ±2 °C.

Preparation of Standards

Reconstitute lyophilized Human GDF15 Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human GDF15 in Master Standard is 800 pg/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 800 pg/mL (lyophilized)	See CofA	800 pg/mL
Std2	300 µL of Std1	300 µL	400 pg/mL
Std3	300 µL of Std2	300 µL	200 pg/mL
Std4	300 µL of Std3	300 µL	100 pg/mL
Std5	300 µL of Std4	300 µL	50 pg/mL
Std6	300 µL of Std5	300 µL	25 pg/mL
Blank		300 µL	0 pg/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:10 in Dilution Buffer, prior to use, see Preparation of samples.

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Preparation of Wash Buffer 1×

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15 × conc. to 700 mL of deionized /distilled water (dH_2O). Mix well. Store at 4 °C for two weeks or at -20 °C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70 °C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:10 for healthy individuals, i.e., for singlets 20 µL of sample + 180 µL of Dilution Buffer, for duplicates 30 µL of samples + 270 µL of Dilution Buffer, respectively.

Recommended dilution of samples is 1:40 for individuals in condition in which is expected higher level of GDF15, i.e., for singlets 5 µL of sample + 195 µL of Dilution Buffer, for duplicates 10 µL of samples + 390 µL of Dilution Buffer, respectively.

Do not store the diluted samples.

Assay procedure

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for 1 hour at 25 °C ±2 °C, shaking at 300 rpm.
3. Wash the wells 3-times with 1× Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for 1 hour at 25 °C ±2 °C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for 30 min at 25 °C ±2 °C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for 20 min at 25 °C ±2 °C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

Performance characteristics

Samples used in the tests were diluted 1:10 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human GDF15 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 6.25 p/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	1649	158	10
2	1696	54	3

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	841	30	4
2	1604	61	4

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		1818	-	-
	2×	825	909	91
	4×	415	454	91
	8×	186	227	82
2		2871	-	-
	2×	1460	1436	102
	4×	711	718	99
	8×	348	359	97

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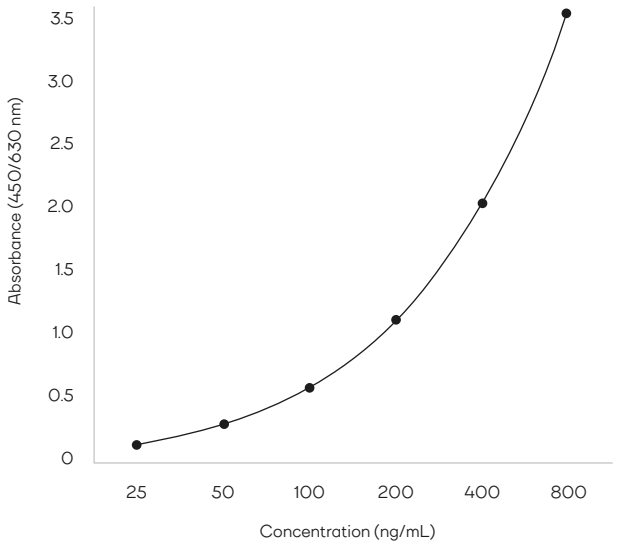
Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	33	-	-
	100	142	133	107
	50	89	83	107
	25	60	58	103

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

Human GDF15 standard curve



Resources

1 Wang D, Day EA, Townsend LK, Djordjevic D, Jørgensen SB, Steinberg GR. GDF15: emerging biology and therapeutic applications for obesity and cardiometabolic disease. Nat Rev Endocrinol. 2021 Oct;17(10):592-607. doi: 10.1038/s41574-021-00529-7. Epub 2021 Aug 11. PMID: 34381196.

2 Rochette L, Dogon G, Zeller M, Cottin Y, Vergely C. GDF15 and Cardiac Cells: Current Concepts and New Insights. Int J Mol Sci. 2021 Aug 18;22(16):8889. doi: 10.3390/ijms22168889. PMID: 34445593; PMCID: PMC8396208.

3 Li S, Ma YM, Zheng PS, Zhang P. GDF15 promotes the proliferation of cervical cancer cells by phosphorylating AKT1 and Erk1/2 through the receptor ErbB2. J Exp Clin Cancer Res. 2018 Apr 10;37(1):80. doi: 10.1186/s13046-018-0744-0. PMID: 29636108; PMCID: PMC5894198.

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