Quantikine® ELISA

Human GDF-15 Immunoassay

Catalog Number DGD150
SGD150
PDGD150

For the quantitative determination of Growth Differentiation Factor 15 (GDF-15) concentrations in cell culture supernates, serum, plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Growth Differentiation Factor-15 (GDF-15), also known as macrophage inhibitory cytokine-1 (MIC-1), placental transforming growth factor-β, prostate-derived factor, and placental bone morphogenetic protein, is a divergent member of the transforming growth factor beta (TGF-β) superfamily (1-3). GDF-15 is initially synthesized as a 40 kDa inactive precursor protein. It is then proteolytically cleaved to release the active C-terminal fragment, which is assembled into a disulfide-linked homodimer of 28 kDa to become biologically active GDF-15 (1, 4). In humans, GDF-15 is predominantly expressed in the placenta, with low levels in the kidney, pancreas, and prostate. However, its expression can be rapidly induced by cytokines such as interleukin-1 and TGF-β (1-3).

GDF-15 has diverse biological functions. Early studies have shown that low serum GDF-15 levels correlate with miscarriages, indicating that it might be able to suppress inflammation in early pregnancy (5, 6). GDF-15 also plays an important role in tumorigenesis and metastasis. It has been observed that in many types of cancers, such as colorectal, breast, and prostate, the expression of GDF-15 is dramatically increased (7-9). Additionally, in cancer patients, serum levels of GDF-15 are elevated, which are of value in disease diagnosis and stratification (10-12). GDF-15 is strongly induced by the tumor suppressor gene p53 and other anti-tumorigenic agents, such as the non-steroidal anti-inflammatory drugs and peroxisome proliferators-activated receptor γ. These findings suggest that GDF-15 may be a downstream target of those signaling pathways that regulate cell cycle arrest and apoptosis (13-15). Through the modulation of neuronal pathways important in the regulation of appetite and energy homeostasis, GDF-15 mediates cancer-induced anorexia and weight loss (16).

GDF-15 has cardioprotective functions. In mouse models, induction of GDF-15 protects the heart from ischemia/reperfusion injury and its over-expression attenuates ventricular dilation and heart failure. Conversely, in GDF-15 gene-targeted mice, reduction of GDF-15 expression results in enhanced cardiac hypertrophic growth (17, 18). In humans, serum GDF-15 concentrations have been shown to be associated with the risk of acute coronary syndrome as well as its prognosis (19, 20). GDF-15 might exert its cardioprotective effects through activation of the PI3K-Akt signaling pathway and the Smad protein (21, 22).

GDF-15 is also involved in iron homeostasis. Tanno *et al*. have reported that in patients with β thalassemia, serum GDF-15 levels are elevated, which results in the suppression of the iron regulatory protein hepcidin. Significant induction of GDF-15 has also been observed in individuals with iron deficiency (23, 24).

The Quantikine® Human GDF-15 Immunoassay is a 3.5 hour solid phase ELISA designed to measure human GDF-15 in cell culture supernates, serum, plasma, and urine. It contains CHO cell-expressed recombinant human GDF-15 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human GDF-15 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values of natural human GDF-15.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human GDF-15 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any GDF-15 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human GDF-15 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of GDF-15 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. 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 Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DGD150	CATALOG # SGD150	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human GDF-15 Microplate	893649	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human GDF-15.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human GDF-15 Conjugate	893650	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human GDF-15 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human GDF-15 Standard	893651	1 vial	6 vials	Recombinant human GDF-15 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-9	895167	1 vial	6 vials	11 mL /vial of a buffered protein solution with preservatives. *May contain a precipitate. Warm to room temperature and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*	
Calibrator Diluent RD5-20 Concentrate	895346	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *Use as a concentrate in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DGD150 contains sufficient materials to run an ELISA on one 96 well plate.
SGD150 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDGD150). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human GDF-15 Controls (optional; R&D Systems®, Catalog # QC21).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & 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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5-20 Concentrate.

Urine samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-20 Concentrate.

REAGENT PREPARATION

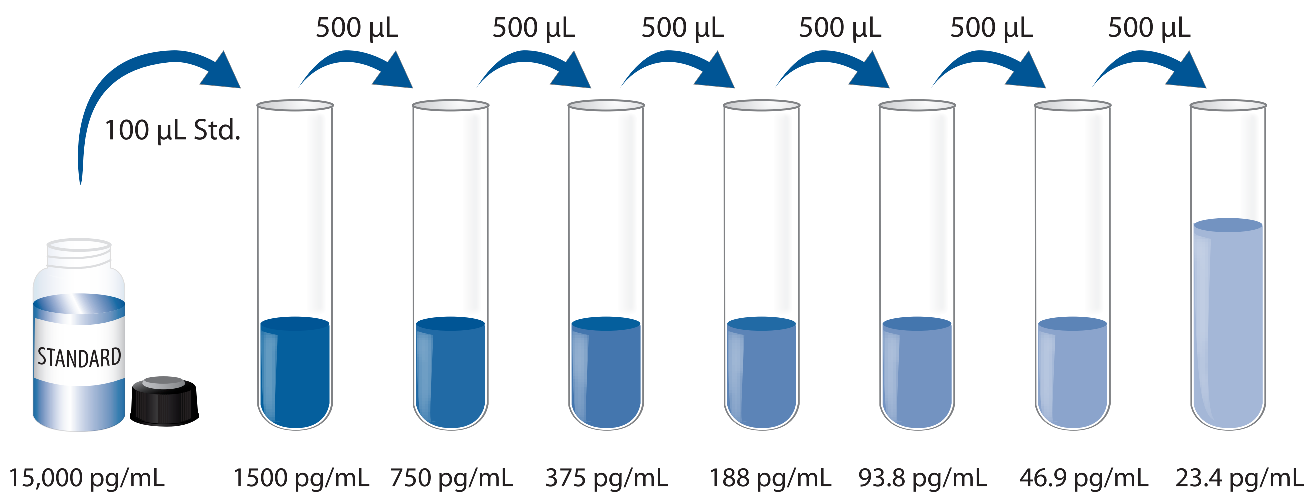
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human GDF-15 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human GDF-15 Standard with Calibrator Diluent RD5-20 Concentrate. This reconstitution produces a stock solution of 15,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-20 Concentrate into the 1500 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 1500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-20 Concentrate serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1-9 to each well. *Assay Diluent RD1-9 may contain a precipitate. Warm to room temperature and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*
4. Add 50 µL of standard, control, or 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.
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 GDF-15 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 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.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

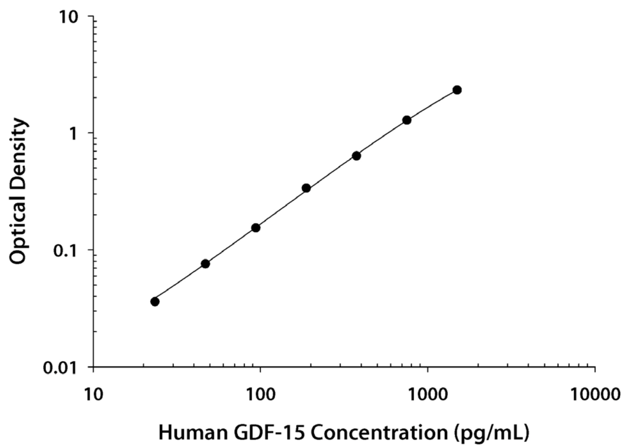
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human 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.

TYPICAL DATA

This 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	0.009 0.010	0.010	—
23.4	0.044 0.047	0.046	0.036
46.9	0.085 0.086	0.086	0.076
93.8	0.161 0.167	0.164	0.154
188	0.341 0.353	0.347	0.337
375	0.632 0.657	0.645	0.635
750	1.280 1.305	1.293	1.283
1500	2.329 2.338	2.334	2.324

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	238	456	886	225	442	900
Standard deviation	4.27	9.86	25.0	13.4	20.6	50.5
CV (%)	1.8	2.2	2.8	6.0	4.7	5.6

RECOVERY

The recovery of human GDF-15 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	94-106%
Serum* (n=4)	98	88-107%
EDTA plasma* (n=4)	97	85-108%
Heparin plasma* (n=4)	96	86-108%
Urine* (n=4)	102	88-108%

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

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human GDF-15 ranged from 0.0-4.4 pg/mL. The mean MDD was 2.0 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human GDF-15 produced at R&D Systems®.

For research use only. Not for use in diagnostic procedures.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human GDF-15 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	102	101	103	101	100
	Range (%)	93-109	100-103	101-106	98-107	98-104
1:4	Average % of Expected	106	102	107	105	103
	Range (%)	96-114	99-104	106-110	104-106	101-104
1:8	Average % of Expected	108	103	108	108	107
	Range (%)	100-115	102-106	105-112	106-115	104-112
1:16	Average % of Expected	108	101	108	110	109
	Range (%)	101-115	99-102	102-112	104-115	105-110

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

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human GDF-15 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=35)	565	337-1060	194
EDTA plasma (n=35)	534	289-1096	214
Heparin plasma (n=35)	507	278-1064	185

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Urine (n=14)	5.71	1.00-20.1	6.08

Cell Culture Supernates:

HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human GDF-15, and measured 46.0 ng/mL.

JEG-3 human epithelial choriocarcinoma cells were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL of streptomycin sulfate for 3 days. An aliquot of the cell culture supernate was removed, assayed for human GDF-15, and measured 17.6 ng/mL.

JAR human choriocarcinoma cells were cultured in DMEM supplemented with 10% FBS until confluent. An aliquot of the cell culture supernate was removed, assayed for human GDF-15, and measured 12.3 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human GDF-15.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human GDF-15 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:	Recombinant mouse:
GDF-9	GDF-1
GDF-11	GDF-3
	GDF-5
	GDF-6
	GDF-7
	GDF-8
	GDF-9

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