

HUMAN GROWTH DIFFERENTIATION FACTOR 15 (GDF-15) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN GDF-15 CONCENTRATIONS IN SERUM
AND PLASMA



FOR RESEARCH USE ONLY. NOT FOR USE IN
DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN GDF-15 ELISA
Catalog No.	SK00108-01
Lot No.	
Formulation	96 T
Standard range	15.625 - 1000 pg/mL
Sensitivity	7.8 pg/mL
Sample Volume	100 μ L
Dilution Factor	2 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Plasma, Serum, Cell Culture Supernates
Specificity	Human GDF-15 only
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2°C - 8°C

Order Contact:

AVISCIERA BIOSCIENCE INC.

2348 Walsh Ave., Suite C

Santa Clara, CA 95051

Tel: (408) 982 0300

Fax: (408) 982 0301

Email: Sales@AvisceraBioscience.com

Info@AvisceraBioscience.com

www.AvisceraBioscience.com

INTRODUCTION

Human GDF-15 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human GDF-15 in cell culture supernates, serum, and plasma. It contains recombinant Human GDF-15 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human GDF-15. Results obtained with naturally occurring GDF-15 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural Human GDF-15.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for 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, a biotinylated polyclonal antibody specific for GDF-15 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, 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.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors

have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
GDF-15 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal purified IgG against human GDF-15.	108-01-01	1 plate
GDF-15 Standard – 500 pg/vial of recombinant human GDF-15 in a buffered protein base with preservatives; lyophilized.	108-01-02	2 vials
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of biotinylated polyclonal purified IgG against human GDF-15 with preservatives; lyophilized.	108-01-03	1 vial
Positive Control – one vial of recombinant human GDF-15, lyophilized	108-01-04	1 vial
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer - 60mL of buffered protein based solution with preservatives	DB01	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution	TMB01	1 bottle
Stop Solution - 11 mL of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate

Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C or -70° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C or -70° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate 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 or -70° C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require a 2-fold dilution. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

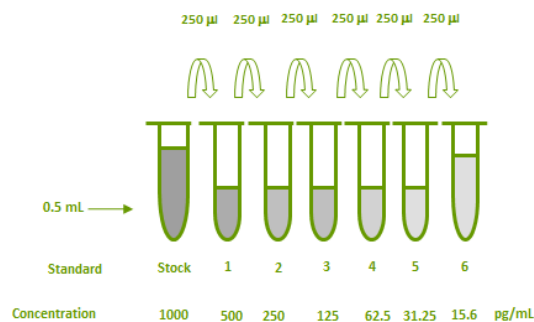
REAGENT PREPARATION

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. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

GDF-15 Standard - Refer to vial label for reconstitution volume. Reconstitute the **GDF-15** standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	0.5mL	1000 pg/ml
# 1	250µl of stock	250µl	500 pg/ml
# 2	250µl of 1	250µl	250 pg/ml
# 3	250µl of 2	250µl	125 pg/ml
# 4	250µl of 3	250µl	62.5 pg/ml
# 5	250µl of 4	250µl	31.25 pg/ml
# 6	250µl of 5	250µl	15.625 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and

transfer 60 μL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

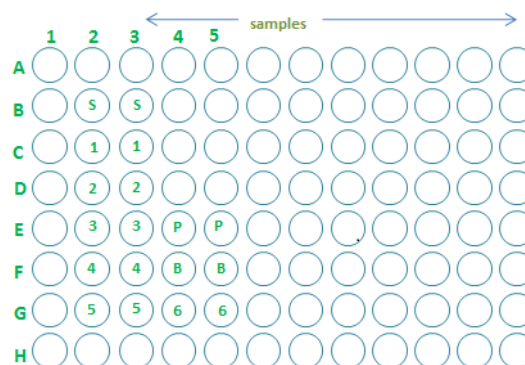
Positive Control - Reconstitute the positive control with 0.5 mL of Dilution Buffer to make positive control solution. **Note:** Positive Control should be used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 μL of **Dilution Buffer** to Blank wells (F4, F5).
4. Add 100 μL of **Standard** (from B2, B3 to G2, G3 and G4, G5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record 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 **1x Wash Buffer** (300 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 **Detection Antibody working solution** to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 μL of **Substrate Solution** to each well. Incubate for 2-5 minutes at room temperature. **Protect from light.**

11. Add 100 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. 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 the graph. The data may be linearized by plotting the log of the 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.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO-expressed recombinant human GDF-15.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of GDF-15 was 7.8 pg/mL.

SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

PROTEINS	CROSS-REACTIVITY
Human GDF-15	100%
Human GDF-11	0%
Mouse GDF-5	0%
Mouse GDF-6	0%
Mouse GDF-7	0%
Mouse GDF-8	0%
Mouse GDF-8pro	0%

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.050)
15.625	0.059
31.25	0.115
62.5	0.219
125	0.423
250	0.766
500	1.337
1000	1.866

LINEARITY

To assess the linearity of the assay, pooled research human EDTA plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
2X	299.462	598.924	100
4X	137.742	550.968	92









To assess the linearity of the assay, pooled research human serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
2X	388.530	777.06	100
4X	155.617	622.468	80.1

REFERENCES

- Staff AC, et al. Growth differentiation factor-15 as a prognostic biomarker in ovarian cancer. *Gynecol Oncol.* 2010 Jun 22. [Epub ahead of print]
- Roth P, et al. GDF-15 Contributes to Proliferation and Immune Escape of Malignant Gliomas. *Clin Cancer Res.* 2010 Jun 9. [Epub ahead of print]
- Chan D, Ng LL. Biomarkers in acute myocardial infarction. *BMC Med.* 2010 Jun 7; 8:34. PubMed PMID: 20529285
- Eggers KM, et al. Improving long-term risk prediction in patients with acute chest pain: the Global Registry of Acute Coronary Events (GRACE) risk score is enhanced by selected nonnecrosis biomarkers. *Am Heart J.* 2010 Jul; 160 (1): 88-94.
- Lajer M, et al. Plasma growth differentiation factor-15 independently predicts all-cause and cardiovascular mortality as well as deterioration of kidney function in type 1 diabetic patients with nephropathy. *Diabetes Care.* 2010 Jul; 33 (7): 1567-72. Epub 2010 Mar 31.
- Taddei S, Viridis A. Growth differentiation factor-15 and cardiovascular dysfunction and disease: malefactor or innocent bystander? *Eur Heart J.* 2010 May; 31 (10): 1168-71
- Eggers KM, et al. Growth-differentiation factor-15 for long-term risk prediction in patients stabilized after an episode of non-ST-segment-elevation acute coronary syndrome. *Cir Cardiovasc Genet.* 2010 Feb 1; 3 (1): 88-96. Epub 2009 Dec 11.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100 μ L of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ L Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ L Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. Protect from light.

Aspirate and wash 4 times.

Add 100 μ L Substrate Solution to each well. Incubate 2-5 min on plate shaker. Protect from light.

Add 100 μ L Stop Solution to each well. Read 450nm within 15 min