

HIGH SENSITIVITY GROWTH DIFFERENTIATION FACTOR-15 (GDF15) (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN GDF15 CONCENTRATIONS IN SERUM AND EDTA PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HIGH SENSITIVITY GDF15 (HUMAN) ELISA KIT
Catalog No.	SK00108-06
Lot No.	
Formulation	96 T
Standard range	6.25 – 400 pg/mL
Sensitivity	2 pg/mL
Sample require	100 µL
Dilution Factor	2~8 (<i>Optimal dilutions should be determined by each laboratory for each application</i>)
Sample Type	Serum, EDTA Plasma
Specificity	Human GDF15
Calibration	Human GDF15 Recombinant (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8°C for 1 month. See page 2-3 for more information.
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This High Sensitivity GDF15 (Human) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human GDF15 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human GDF15 and monoclonal antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural GDF15 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human GDF15. The capture antibody can bind to the human GDF15 in the standard and samples. After washing the plate of any unbound substances, a monoclonal antibody HRP Conjugated against human GDF15 is added to the wells. After the last wash to remove any unbound Antibody enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human GDF15 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

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

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix 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.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
GDF15 Microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified antibody against human GDF15.	108-06-01	1 plate
GDF15 Standard – refer to lot of recombinant human GDF15 in a buffered protein base with preservative; lyophilized.	108-06-02	1 vial
Detection Antibody HRP – refer to lot of vial, 100-fold concentrate of a monoclonal antibody HRP Conjugated against soluble human GDF15 with preservative; lyophilized.	108-06-03	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB10B	1 bottle
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution – 11mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution - 11 mL of 0.5M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 1 month. For longer storage for up to 10 months, unopened Standard and Dilution Buffer should be stored at - 20°C. Detection Antibody-HRP Conjugate and TMB Substrate Solution should be stored only at 2 ~ 8 °C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

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 $\leq -20^{\circ}\text{C}$. 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples may require 2 ~ 8-fold dilution. A suggested 2-fold dilution is 125 μL sample + 125 μL Dilution Buffer. A suggested 4-fold dilution is 60 μL of sample + 180 μL Dilution Buffer. A suggested 8-fold dilution is 30 μL of sample + 210 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 1x Wash Buffer.

GDF15 Standard - Reconstitute the GDF15 standard with refer to lot of Dilution Buffer (DB10B). Pipette 250 μL of Dilution Buffer into tubes #2 to #7. Use the 400 pg/mL high standard solution to produce a dilution series (below). Mix each tube thoroughly

before the next transfer. The 400 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	Refer to lot	Refer to lot
# 1	Refer to lot	Refer to lot	400 pg/ml
# 2	250 μL of 1	250 μL	200 pg/ml
# 3	250 μL of 2	250 μL	100 pg/ml
# 4	250 μL of 3	250 μL	50 pg/ml
# 5	250 μL of 4	250 μL	25 pg/ml
# 6	250 μL of 5	250 μL	12.5 pg/ml
# 7	250 μL of 6	250 μL	6.25 pg/ml

Detection Antibody Concentrate - Pipette 10.395 mL of Dilution Buffer (DB10B) into a 15 mL centrifuge tube and transfer 105 μL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Detection Antibody-HRP Conjugate should be used within a few hours (**protect from light**).

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 μL per well of Dilution Buffer to Blank wells.
4. Add 100 μL of Standard dilutions in reverse order of serial dilution #7 - #1, samples or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 HRP working solution to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
9. 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.
10. Determine the optical density of each well within 3 minutes, using a microplate 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 or 4-parameter curve fit.

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

Calculation of samples with a concentration exceeding that of standard 400 pg/ml may result in inaccurate, low human GDF15 levels. Such samples require further external predilution according to expected human GDF15 values with Dilution Buffer in order to precisely quantify the actual human GDF15 level.

SPECIFICITY

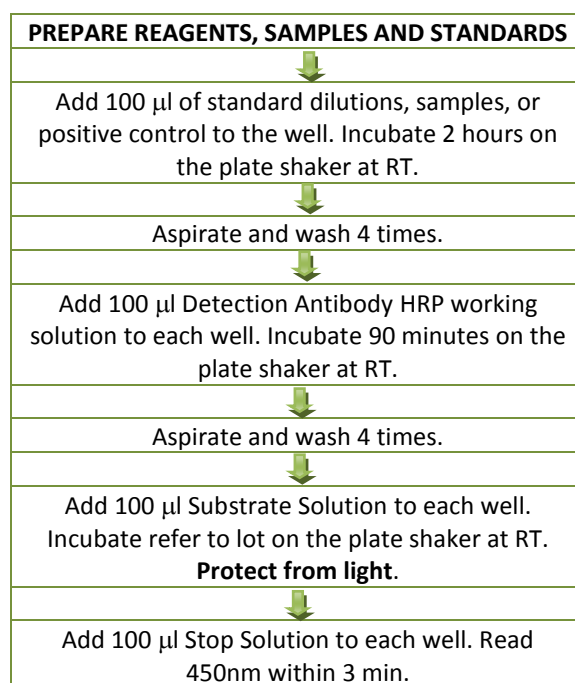
PROTEIN NAME	CROSS-REACTIVITY
Human GDF15	100%
Human GDF11	0
Human GDF8	0

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (refer to lot)
6.25	0.039
12.5	0.091
25	0.169
50	0.327
100	0.678
200	1.210
400	2.419

SUMMARY OF ASSAY PROCEDURE



The research samples were diluted by Dilution Buffer (DB10B). Its linearity and recovery was assayed by High Sensitivity GDF15 (H) ELISA Kit SK00108-06

Sample Type	Dilution Factor	Assayed (pg/mL)	Final (pg/mL)	Recovery (%)
Human Serum	2 X	294.532	589.064	100
Human Serum	4 X	148.329	593.316	101
Human Serum	8 X	72.916	583.328	99
Human Plasma	2 X	263.391	527.183	100
Human Plasma	4 X	129.074	516.296	98
Human Plasma	8 X	67.241	537.928	102