

Human GDF15 ELISA Kit

Sandwich ELISA

Catalog EL-213-74615

INTRODUCTION

GDF15 is a stress-induced hormone that, despite belonging structurally to the TGF-beta superfamily, signals through its own dedicated receptor system: circulating GDF15 binds GFRAL, a receptor expressed on neurons in the brainstem area postrema and nucleus tractus solitarius, which in turn engages the RET co-receptor to trigger downstream ERK signaling. This brainstem circuit converts cellular stress signals -- tissue injury, hypoxia, inflammation, oxidative stress, or drug exposure -- into an aversive response marked by nausea, reduced appetite, and weight loss. GDF15 is synthesized as a precursor that is proteolytically cleaved into a disulfide-linked mature dimer, and elevated circulating levels have been documented in cancer-associated cachexia, cardiovascular and metabolic stress states, and pregnancy, where high early-pregnancy levels are linked to the severity of nausea and vomiting, including hyperemesis gravidarum.

PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format in which the microtiter plate wells are pre-coated with a capture antibody specific to Human GDF15. Standards or samples are added to the appropriate wells, followed by an HRP-conjugated detection antibody also specific to Human GDF15. Upon addition of TMB substrate solution, only wells containing the target analyte bound to the HRP-conjugated antibody produce a colorimetric signal. The enzymatic reaction is stopped by the addition of sulfuric acid stop solution, and absorbance is measured at 450 nm (± 10 nm). Sample concentrations are determined by comparing the optical density of each sample against a standard curve generated from the provided GDF15 standards.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard Microplate	2 strips x 8 wells
HRP Conjugate Antibody (100×)	70 µL
Standard/Sample Diluent Buffer	24 mL
HRP Conjugate Diluent	10 mL
Wash Buffer (25×)	24 mL
TMB Substrate Solution	12 mL
Stop Reagent	7 mL
Plate Covers	2 pieces
Instruction Manual	1
<i>Do not mix or substitute reagents with those from other lots.</i>	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient

sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the HRP-conjugated detection antibody concentrate 100-fold with HRP Conjugate Diluent, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 3000 down to 46.88 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (μL)	Cal. Diluent (μL)	Final Vol (μL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	3000
Std 2	Std 1	500	500	1000	1500
Std 3	Std 2	500	500	1000	750
Std 4	Std 3	500	500	1000	375
Std 5	Std 4	500	500	1000	187.5
Std 6	Std 5	500	500	1000	93.75
Std 7	Std 6	500	500	1000	46.88
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 50 μL Standard/Sample Diluent Buffer to each Standard well, and add 50 μL sample to the sample well. Immediately add 50 μL 1× HRP Conjugate Antibody Working Solution to each well. Incubate at 37°C on Microplate oscillator for 60 min.

2. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.

3. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

- Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
- The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
- Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 5.22 pg/mL

Recovery: Serum (n = 5): 87-99% (avg 94%); EDTA plasma (n = 5): 84-95% (avg 89%)

**88%); Heparin plasma (n = 5): 82-97%
(avg 92%)**

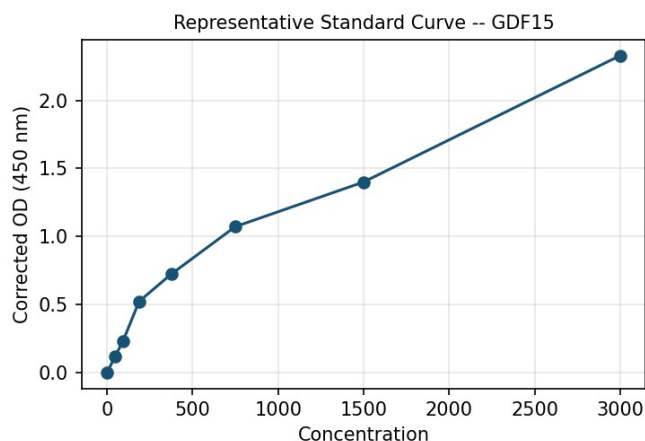
Specificity: This assay has high sensitivity and excellent specificity for detection of Human GDF15. No significant cross-reactivity or interference between Human GDF15 and analogues was observed.



This kit employs a sandwich enzyme immunoassay technique to quantify Human GDF15 in biological samples.

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SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.