Total GDF-15 ELISA

AL-1014-r

RUO

INTENDED USE

The Total GDF-15 enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of human GDF-15 in human serum, urine, and other biological fluids. The assay detects total GDF-15 including histidine 202 to aspartate mutation (HH, HD & DD variant) in the mature domain. The assay is intended for invitro Research Use Only.

SUMMARY AND EXPLANATION

Growth/differentiation Factor 15 (GDF-15, also called as MIC-1, NAG-1 and NRG-1, Uniprot: Q99988) is a divergent member of the TGF-β superfamily of growth factors. It is encoded in humans by a gene in chromosome 19. The human GDF-15 gene encodes for a protein of 308 amino acid residues which consists of a signal sequence (residues 1-29), pro-domain (30-194) and mature growth factor domain (195-308). The protein is secreted from the producing cell and the precursor containing the pro- and mature domains is proteolytically processed by furin-like protease, typically in the Golgi complex, but sometimes also an unprocessed protein is secreted. Two mature domains dimerize forming a typical TGF-β-like structure with four b-strands and an a-helix in each protomer, with an interfacial disulfide stabilizing the mature growth factor. The molecular weight of a mature GDF-15 dimer is 25 kDa.

Mature GDF-15 has its dedicated GFRAL transmembrane receptor which is found only in very restricted area in the hindbrain. GFRAL facilitates GDF-15 signaling through Ret receptor tyrosine kinase, similar to GDNF subfamily of growth factors.

Approximately 25% of humans have a missense polymorphism in GDF-15 gene resulting in mutation of histidine 202 to aspartate (histidine 6 in the mature domain), close to the N-terminus of the mature growth factor. This variant is associated with phenotypes in prostate cancer, hyperemesis gravidarum, (severe morning sickness in pregnancy) and rheumatoid arthritis. The underlying mechanism and significance of these associations is still unclear though.

GDF-15 expression in healthy subjects is abundant in placenta, followed by the prostate and very low levels in the bladder, kidney, colon, stomach, liver, gall bladder, pancreas, and endometrium[1-3]. GDF-15 is expressed by cardiomyocytes, adipocytes, macrophages, endothelial and vascular smooth muscle cells[4]. High circulating GDF-15 concentration are in general related to inflammation, myocardial ischemia, and cancer except for in pregnancies. It is often induced under stress to maintain cell and tissue homeostasis[5-6].

GDF-15 is proposed as a diagnostic biomarker in colorectal[7-8], ovarian[9], early-stage lung cancer[8,10]. It is shown to be an accurate marker for differentiating pancreatic adenocarcinoma and chronic pancreatitis[11]. It has also shown to be a potential biomarker to aid in the discrimination between prostate cancer and benign hyperplasia[12-14], studied as a biomarker for disease prognosis and as an emerging target for cancer immunotherapy[15]. Neutralizing antibodies against GDF-15 has been studied to revert the weight loss in animal models of cancer-related cachexia[15-17].

PRINCIPLE OF THE TEST

The total GDF-15 ELISA is a quantitative three-step sandwich type immunoassay that is designed to measure human GDF-15. The antibodies used in this assay also detects the missense polymorphism in GDF-15 gene resulting in mutation histidine 202 to aspartate mutation in mature domain along with the native GDF-15 molecule. In the first step Calibrators, Controls and unknown samples and assay buffer solution are added to antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated GDF-15 antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution After the second incubation and washing, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the GDF-15 antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of GDF-15 in the samples and calibrators.
Note: *Use of this kit in combination with the GDF-15 H-specific assay (DD non-detectable) ELISA can help estimate the mutant (DD) concentration in serum as the AL-1018-r does not detect DD variant.*

MATERIALS SUPPLIED

CAL-1014A GDF-15 Calibrator A/Sample Diluent

One vial, 8mL, labeled GDF-15 Calibrator A/Sample Diluent, containing 0 pg/mL GDF-15 in serum with non-mercury preservative. Store unopened at 2-8°C until the expiration date.

CAL-1014B - CAL-1014F GDF-15 Calibrators B-F (Lyophilized)

Five vials, labeled B-F containing approximate concentrations of 45.0 – 2900.0 pg/mL GDF-15 in serum with non-mercury preservative. Refer to calibration card for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze for multiple use.
NOTE: *The calibrators are traceable to Recombinant Human GDF-15 from R & D Systems (Biotechne, USA).*

CTR-1014-I & CTR-1014-II GDF-15 Controls

Two vials, labeled Levels I and II containing low and high GDF-15 concentrations in serum with non-mercury preservative. Refer to calibration card for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze for multiple use.

PLT-1014 Total GDF-15 Antibody Coated Microtitration strips

One strip holder, containing 12 strips and 96 microtitration wells with GDF-15 antibody immobilized to the inside wall of each well. Store at 2 to 8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

ASB-1014 Total GDF-15 Assay Buffer

One bottle, 12 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

BCR-1014 Total GDF-15 Biotin Conjugate Ready-to-Use

One bottle, 12 mL each, containing anti GDF-15 antibody in a protein buffer with a non-mercury preservative. Store at 2 to 8°C until the expiration date.

SAR-1014 Total GDF-15 Streptavidin-Enzyme Conjugate Ready-To-Use (RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

TMB-100 TMB Chromogen Solution

One bottle, 12 mL each, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2 to 8°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

STP-100 Stopping Solution

One bottle, 12 mL each, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405 nm, and 630 nm.
2. Microtitration orbital plate shaker.
3. Microtitration plate washer.
4. Semi-automated/manual precision pipette to deliver 2 µL–100 µL.
5. Repeater pipette
6. Vortex mixer.
7. Deionized water.
8. 12 x 75 mm culture tubes.
9. Tight fitting 12 x 75 mm tube racks.

WARNINGS AND PRECAUTIONS

For *Research Use Only.*

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

WARNING: Potential Biohazardous Material

This reagent may contain some heat treated human and animal source material (e.g., serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007[18]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 as a preservative. Pro-Clean 400 and peroxide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) Serum is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Samples may be stored at 4°C if assayed within 24 hours; otherwise, samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

d) Avoid assaying lipemic, hemolyzed or icteric samples.

e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.

f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.[19]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Total GDF-15 ELISA assay. It is the responsibility of the customer to validate the assay for their use. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix different lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells accurately and efficiently to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **GDF-15 Calibrators B-F and Controls I & II**: Tap and reconstitute GDF-15 Calibrator B-F and controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
   Note: For increased sensitivity, dilute reconstituted Calibrator B as suggested below.
   CAL B/3: Mix 50 µL of reconstituted Calibrator B with 100 µL of Calibrator A/Sample diluent in an Eppendorf tube and vortex gently.
2. **Wash Solution:** Dilute Wash Concentrate A 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

SAMPLE PREPARATION

1. Maternal serum specimens should be diluted 1:15 in Calibrator A/Sample Diluent (5uL of sample in 70µL of diluent) and recommended to be tested on the same day of dilution.

Note: *The Calibrator A /Sample Diluent for AL-1014-r (CAL-1014A) and AL-1018-r (CAL-1018A) have identical formulation. If GDF-15 (H-specific) ELISA (AL-1018-r) assay is being performed along with the Total GDF-15 ELISA (AL-1018-r), the same preparation of diluted sample can be used in both assays.*

Maternal urine specimens should be diluted **1:50** in calibrator A/Sample Diluent. Urine specimens may require a **two-step** dilution.

STEP ONE:

1. For each unknown urine specimen, label one vial.
2. Add 2µL of the specimen to each vial.
3. Add 8µL of Calibrator A/Sample Diluent to each vial.

STEP TWO:

4. Add 45µL of Calibrator A/Sample Diluent to each vial
5. Add 5µL of the diluted urine specimens from first step to pre-label vial and vortex well.
6. The sample is now ready for analysis.

ASSAY PROCEDURE

Allow all samples and reagents to reach room temperature and mix reagents thoroughly by gentle inversion before use. Calibrators, controls, and samples should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Pipette **10 µL** of the **Calibrators, Controls** and **Unknowns** to the designated calibrator, control, and unknown wells. *For increased sensitivity, **add calibrator B/3** to the designated well (see Preparation of Reagents).*
3. Add **100 µL** of the Total GDF-15 Assay Buffer to **all wells** using a repeater pipette.
4. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **45 minutes** at room temperature (23 ± 2°C).
5. Aspirate and wash each well **5 times** with the wash solution **(350 µL/well)** using an automatic microplate washer.
6. Add **100 µL** of the Total GDF-15 Biotin Conjugate ready-to-use solution to each well using a repeater pipette.
7. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
8. Aspirate and wash each well **5 times** with the wash solution **(350 µL/well)** using an automatic microplate washer.
9. Add **100 µL** of the Total GDF-15 Streptavidin Conjugate ready-to-use solution to each well using a repeater pipette.
10. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **20 minutes** at room temperature (23 ± 2°C).
11. Aspirate and wash each well **5 times** with the wash solution **(350 µL/well)** using an automatic microplate washer.
12. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
13. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 minutes** at room temperature (23 ± 2°C).
    ***NOTE:** Visually monitor the color development to optimize the incubation time.*
14. Add **100 µL** of the **Stopping solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm.**
    ***NOTE**: Zero calibrator should be programmed as "**Blank**" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at **450 nm** with background wavelength correction at **630 nm**.*

RESULTS

***NOTE:** The results in this package insert were calculated by plotting the **log optical density (OD) data on the y-axis and log GDF-15 concentration on X-axis** using a cubic regression curve fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.*

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Optimum results can be obtained at incubation temperature of (23 ± 2°C).
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the GDF-15 concentrations in pg/mL along the x-axis, using a cubic regression curve fit.
4. Determine the GDF-15 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding GDF-15 concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately further diluted with the 0 pg/mL (Cal. A) and re-assayed. For diluted samples multiply the result by the dilution factor.
6. Any sample reading lower than the LoD should be reported as such.

LIMITATIONS

1. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.
2. As for any assay employing antibodies, the possibility exists for interference by heterophile antibodies in the samples[20]. Interference from heterophile antibodies has not been evaluated for this assay.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Kit controls or other commercial controls should fall within established confidence limits.
- The confidence limits for kit controls are printed on the **calibration card**.
- A full calibration curve, along with low- and high-level controls, should be included in each assay.
- TMB chromogen solution should be colorless. Development of a blue color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean (O.D)	Conc. (pg/mL)
A1, A2	A	0.009 (Blank)	0
B1, B2	B/3	0.020	15.0
C1, C2	B	0.066	45.0
D1, D2	C	0.22	175.0
E1, E2	D	0.65	490.0
F1, F2	E	1.46	1227.0
G1, G2	F	3.06	2906.0

***CAUTION:** The above data must not be employed in lieu of data obtained by the user in the laboratory.*

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in pg/mL.

Analytical Sensitivity

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 pg/mL) and calibrator B (45.0 pg/mL) is 2.2 pg/mL.

Imprecision

Reproducibility of the Total GDF-15 ELISA assay was determined in a study using kit controls and one serum pool. The study included a total of 6 assays, three replicates of each per assay (n=18). Representative data were calculated and are presented in the following table.

Sample	Mean Conc. (pg/mL)	Within run		Between run		Total	
		SD	CV	SD	CV	SD	CV
Control I	184.0	4.3	2.3%	4.5	2.5%	6.2	3.4%
Control II	482.7	18.8	3.9%	21.1	4.4%	28.2	5.8%
Pool-1	1220.0	52.6	4.3%	19.8	1.6%	56.2	4.6%

Linearity

Multiple dilutions of calibrator F and two serum samples containing various GDF-15 levels were performed in Calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution	Expected (pg/mL)	Observed (pg/mL)	% Recovery	Average % Recovery
Calibrator F	Neat	2906.0			99%
	1:2	1453.0	1460.5	101%	
	1:4	726.5	732.5	101%	

	1:8	363.3	365.4	101%	
	1:16	181.6	178.2	98%	
	1:32	90.8	86.3	95%	
S1	Neat	483.5			104%
	1:2	241.7	246.0	102%	
	1:4	120.9	126.6	105%	
	1:8	60.4	63.8	106%	
S2	Neat	425.1			98%
	1:2	212.6	212.0	100%	
	1:4	106.3	107.9	102%	
	1:8	53.1	49.4	93%	

Recovery

Two serum samples containing low levels of endogenous GDF-15 were spiked with recombinant GDF-15 (2906.0 pg/mL) at two levels (5% and 10%). The concentration of GDF-15 was measured before and after the addition of recombinant GDF-15 and the percentage recovery was calculated.

Sample ID	Endogenous Value (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	% Recovery	Average % Recovery
1	366.3	493.3	498.0	101%	102%
		620.3	642.4	104%	
2	572.1	688.8	735.6	107%	110%
		805.5	919.9	114%	

Species Immunoreactivity

The antibody pair used in the assay ***detects Bovine Follicular Fluid, Monkey, and Porcine*** samples and it ***does not detect Canine, Equine, Ovine, Rabbit, Goat, and Mouse species***.

Specificity

The Total GDF-15 ELISA assay when tested on synthetic dimers of human GDF-15 homozygote for wild type (HH), heterozygote HD and the homozygous mutation (DD) detects all forms almost equally. A combination of Ansh Labs AL-1014-r and AL-1018-r [GDF-15 (H-specific) ELISA] can help estimate the mutant (DD) concentration in serum as the AL-1018-r does not detect DD variant. Neurturin and GDNF (Glial Cell Line-derived Neurotrophic factor) when tested at higher physiological dose were below the detection limit of the assay.

GDF-15 (aa 202)	Expected GDF-15 Concentration (pg/mL)	Observed GDF-15 Concentration (pg/mL)	% Recovery	% Average Recovery
HH/Wild Type	500	529.9	106.0	99.5
	50	46.5	93.1	
HD, DH, HH and DD/ Hetero dimer	500	616.6	123.3	115.5
	50	53.9	107.7	
DD/H202D variant homozygous variant	500	540.0	108.0	96.4
	50	42.4	84.8	
GDNF (Glial Cell Line-derived Neurotrophic factor)	1000	0	ND	ND
Neurturin	1000	0	ND	ND

ND: Not detectable

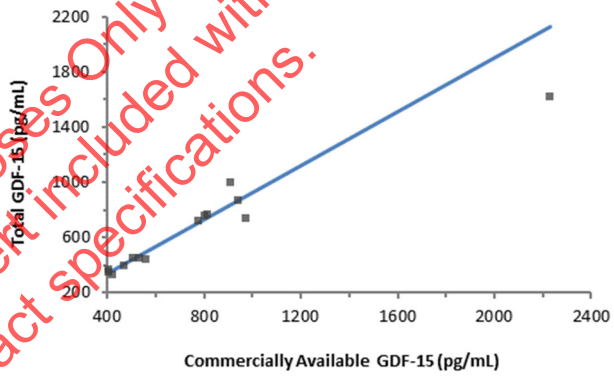
Interference

When Hemoglobin, Biotin, Intralipids and Bilirubin were added at a greater than two folds of their physiological concentration to control samples, GDF-15 average concentration were within ± 10% of the control as represented in the following table.

Interferent	Interferent Dose	Sample ID	Sample GDF-15 (pg/mL)	Dosed Sample GDF-15 (pg/mL)	% Difference	Average % Difference
Hemoglobin	1 mg/mL	1	498.23	549.85	10.4	6.8
		2	557.68	576.11	3.3	
	0.5 mg/mL	1	329.85	335.06	1.6	0.4
		2	357.42	354.43	-0.8	
Biotin	1200 ng/mL	1	523.76	554.17	5.8	7.1
		2	569.58	616.83	8.3	
	600 ng/mL	1	343.99	342.50	-0.4	-0.8
		2	363.03	358.91	-1.1	
Intralipids	20 mg/mL	1	495.20	561.60	13.4	6.9
		2	585.16	587.92	0.5	
	10 mg/mL	1	348.46	342.50	-1.7	0.1
		2	364.52	371.64	2.0	
Bilirubin	0.66 mg/mL	1	381.79	413.80	8.4	4.0
		2	576.11	574.14	-0.3	
Bilirubin	0.2 mg/mL	1	258.27	264.87	2.6	1.8
		2	348.09	351.82	1.1	

Method Comparison

The Total GDF-15 ELISA has been compared to a Commercial GDF-15 assay using 14 serum samples (HH, wild type) in the range of 400-2500 pg/mL.
Passing Bablok analysis of the results yielded the following regression:
Total GDF-15 ELISA (AL-1014-r) = 0.98 (Commercial GDF-15) – 47.0
(r=0.97)



The GDF-15 (H-specific) ELISA (AL-1018-r) has been compared to Total GDF-15 ELISA (AL-1014-r) using 14 serum samples (HH, wild type).
Passing Bablok analysis of the results yielded the following regression:
GDF-15 (H-specific) ELISA (AL-1018-r) = 1.02 Total GDF-15 ELISA (AL-1014-r) – 45.9, (r=0.99)

Expected Value

Expected GDF-15 concentration for ostensibly healthy males, females, pregnant females (1st and 2nd trimester) were calculated using Total GDF-15 ELISA. The expected ranges were calculated using Analyse-It® for Microsoft Excel and are shown in table below.

Sample	n	Median (pg/mL)	GDF-15 Range (pg/mL)
Male	18	847.9	377.0 – 4316.0
Female	19	1182.4	347.2 – 2474.2
1st Trimester	20	12759.7	7168.9 – 25597.0
2nd Trimester	20	12951.2	8851.8 – 29683.9

Note: It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

REFERENCES

1. Kamiya R, Asakura S. Helical transformations of *Salmonella* flagella *in vitro*. *J Mol Biol*. (1976) 106:167–86. doi: 10.1016/0022-2836(76)90306-5
2. Nazarova NY, Chikhirzhina GI, Tuohimaa P. Calcitriol induces transcription of the placental transforming growth factor β gene in

prostate cancer cells via an androgen-independent mechanism. *Mol Biol.* (2006) 40:72–6. doi: 10.1134/S0026893306010110.
3. Uhlen M, Fagerberg L, Hallstrom BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Tissue-based map of the human proteome. *Science*. (2015) 347:1260419. doi: 10.1126/science.1260419.
4. Tsai VWW, Husaini Y, Sainsbury A, Brown DA, Breit SN. The MIC-1/GDF15-GFRAL pathway in energy homeostasis: implications for obesity, cachexia, and other associated diseases. *Cell Metab*. (2018) 28:353–68. doi: 10.1016/j.cmet.2018.07.018
5. Adela R, Banerjee SK. GDF-15 as a target and biomarker for diabetes and cardiovascular diseases: a translational prospective. *J Diabetes Res*. (2015) 2015:490842. doi: 10.1155/2015/490842
6. Guenancia C, Kahli A, Laurent G, Hachet O, Malapert G, Grosjean S, et al. Pre-operative growth differentiation factor 15 as a novel biomarker of acute kidney injury after cardiac bypass surgery. *Int J Cardiol*. (2015) 197:66–71. doi: 10.1016/j.ijcard.2015.06.012.
7. Li C, Wang J, Kong J, Tang J, Wu Y, Xu E, et al. GDF15 promotes EMT and metastasis in colorectal cancer. *Oncotarget*. (2016) 7:860–72. doi: 10.18632/oncotarget.6205.
8. Wang X, Yang Z, Tian H, Li Y, Li M, Zhao W, et al. Circulating MIC-1/GDF15 is a complementary screening biomarker with CEA and correlates with liver metastasis and poor survival in colorectal cancer. *Oncotarget*. (2017) 8:24892–901. doi: 10.18632/oncotarget.15279.
9. Zhao D, Wang X, Zhang W. GDF15 predict platinum response during first-line chemotherapy and can act as a complementary diagnostic serum biomarker with CA125 in epithelial ovarian cancer. *BMC Cancer*. (2018) 18:328. doi: 10.1186/s12885-018-4246-4.
10. Liu YN, Wang XB, Wang T, Zhang C, Zhang KP, Zhi XY, et al. Macrophage inhibitory cytokine-1 as a novel diagnostic and prognostic biomarker in stage I and II nonsmall cell lung cancer. *Chin Med J*. (2016) 129:2026–32. doi: 10.4103/0366-6999.189052.
11. Hogendorf P, Durczynski A, Skulimowski A, Kumor A, Poznanska G, Strzelczyk J. Growth differentiation factor (GDF-15) concentration combined with Ca125 levels in serum is superior to commonly used cancer biomarkers in differentiation of pancreatic mass. *Cancer Biomark*. (2018) 21:505–11. doi: 10.3233/CBM-170203.
12. Gronberg H, Adolfsson J, Aly M, Nordstrom T, Wiklund P, Brandberg Y, et al. Prostate cancer screening in men aged 50-69 years (STHLM3): a prospective population-based diagnostic study. *Lancet Oncol*. (2015) 16:1667–76. doi: 10.1016/S1470-2045(15)00361-7.
13. Li J, Veltri RW, Yuan Z, Christudass CS, Mandecki W. Macrophage inhibitory cytokine 1 biomarker serum immunoassay in combination with PSA is a more specific diagnostic tool for detection of prostate cancer. *PLoS ONE*. (2015) 10:e0122249. doi: 10.1371/journal.pone.0122249.
14. Bansal N, Kumar D, Gupta A, Chandra D, Sankhwar SN, Mandhani A. Relevance of MIC-1 in the era of PSA as a serum based predictor of prostate cancer: a critical evaluation. *Sci Rep*. (2017) 7:16824. doi: 10.1038/s41598-017-17207-2.
15. Lerner L, Tao J, Liu Q, Nicoletti R, Feng B, Krieger B, et al. MAP3K11/GDF15 axis is a critical driver of cancer cachexia. *J Cachexia Sarcopenia Musc*. (2016) 7:467–82. doi: 10.1002/jcsm.12077
16. Chrysovergis K, Wang X, Kosak J, Lee SH, Kim JS, Foley JF, et al. NAG-1/GDF-15 prevents obesity by increasing thermogenesis, lipolysis and oxidative metabolism. *Int J Obes.* (2014) 38:1555–64. doi: 10.1038/ijo.2014.27
17. Tran T, Yang J, Gardner J, Xiong Y. GDF15 deficiency promotes high fat diet-induced obesity in mice. *PLoS ONE*. (2018) 13:e0201584. doi: 10.1371/journal.pone.0201584
18. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
19. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
20. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

This assay is intended for research use only.

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