Bovine GDF-15 ELISA

AL-1021

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INTENDED USE

The Bovine GDF-15 enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of GDF-15 in bovine serum. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

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.

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]. The circulating GDF15 levels in cows and its biological role has not been explored.

PRINCIPLE OF THE TEST

The Bovine GDF-15 ELISA is a quantitative three-step sandwich type immunoassay that is designed to measure Bovine GDF-15. In the first step Calibrators, Controls and unknown samples and assay buffer 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 third 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.

MATERIALS SUPPLIED

CAL-1021A **Bovine GDF-15 Calibrator A/Sample Diluent**

One vial, 3mL, labeled Bovine GDF-15 Calibrator A/Sample Diluent, containing 0 pg/mL Bovine GDF-15 in protein-based buffer and ProClin 300. Store unopened at 2-8°C until the expiration date

CAL-1021B - CAL-1021F **Bovine GDF-15 Calibrators B-F (Lyophilized)**

Five vials, labeled B-F containing approximate concentrations of 4.0 –1000.0 pg/mL Bovine GDF-15 in protein-based buffer and ProClin 300. 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 Bovine GDF-15 preparation.*

CTR-1021-I & CTR-1021-II **Bovine GDF-15 Controls I and II (Lyophilized)**

Two vials, labeled Levels I and II containing low and high Bovine GDF-15 concentrations in protein-based buffer and ProClin 300. 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-1021 **Bovine GDF-15 Antibody Coated Microtitration strips**

One strip holder, containing 12 strips and 96 microtitration wells with Bovine 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-1021 **Bovine GDF-15 Assay Buffer**

One bottle, 10 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

BCR-1021 **Bovine 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-1021 **Bovine 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[7].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain ProClin 300 and Sodium azide[8] as a preservative. ProClin 300 and Sodium azide 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[9].

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the Bovine 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 kit components 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. **Bovine GDF-15 Calibrators B-F and Controls I & II**: Tap and reconstitute Bovine GDF-15 Calibrator B-F and Controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
2. **Wash Solution:** Dilute Wash Concentrate 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 it from moisture.

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. Label the microtitration strips to be used.
2. Pipette **25 µL** of the **Calibrators, Controls** and **Unknowns** to the designated calibrator, control, and unknown wells.
3. Add **75 µL** of the Bovine 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 Bovine 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 Bovine 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 Bovine 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 Bovine GDF-15 concentrations in pg/mL along the x-axis, using a cubic regression curve fit.
4. Determine the Bovine GDF-15 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding Bovine 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 lowest calibrator 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[10]. 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	0.059 (Blank)	0
B1, B2	B	0.026	3.8
C1, C2	C	0.087	13.0
D1, D2	D	0.310	45.6
E1, E2	E	1.165	197.0
F1, F2	F	3.135	824.0

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 (3.9 pg/mL) is 0.42 pg/mL.

Imprecision

Based on NCCLS EP-10 guidelines reproducibility of the Bovine GDF-15 ELISA assay was determined in a study using three samples. The study included a total of 5 assays, 3 replicates each, per assay (n=15). Representative data were calculated and are presented in the following table.

Sample	Mean Conc. (pg/mL)	Total SD (pg/mL)	Total CV %
Low	31.30	2.82	9.02
Mid	96.00	4.51	4.69
High	410.19	42.23	10.30

Linearity

Calibrator F and two samples containing various Bovine GDF-15 levels were serially diluted in Bovine GDF-15 Calibrator A/Sample diluent (CAL-1021A). The % recovery on individual samples is represented in the following table.

Sample ID	Dilution factor (1 in X)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	%Recovery
Calibrator F	Neat	824.00		
	2	412.00	391.41	95%
	4	206.00	191.28	93%
	8	103.00	92.04	89%
	16	51.50	47.83	93%
	32	25.75	24.98	97%
S1	2	837.25		
	4	418.62	421.17	101%
	8	209.31	217.21	104%
	16	104.66	105.82	101%
	32	52.33	52.76	101%
	128	26.16	28.11	107%
S2	Neat	871.16		
	2	435.58	439.35	101%
	4	217.79	218.40	100%
	8	108.90	115.36	106%
	16	54.45	61.04	112%
	32	27.22	29.80	109%

Alternatively, samples containing high levels of Bovine GDF-15 can be diluted in samples containing low levels of Bovine GDF-15 for sample-to-sample dilution as shown in table below. The concentration of sample used as diluted is subtracted from the observed concentration to obtain recovered concentration.

Diluent Conc. (pg/mL)	Sample	Dilution Factor (1 in X)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	Recovered Conc. (pg/mL)	% Recovery
17.36	S1	2	731.47			
		4	365.73	363.64	346.29	94.7
		8	182.87	196.33	178.98	97.9
		16	91.43	108.66	91.30	99.9
		32	45.72	63.15	45.80	100.2
		64	22.86	41.24	23.88	104.5
		128	11.43	29.87	12.52	109.5

Recovery

Two serum samples containing low levels of endogenous Bovine GDF-15 were spiked with sample containing high level of Bovine GDF-15 (1424.18 pg/mL) at three levels (5%, 10%, and 15%). The concentration of Bovine GDF-15 was measured before and after the addition of exogenous Bovine GDF-15 and the percentage recovery was calculated.

Sample ID	Endogenous Value in pg/mL	Expected in pg/mL	Observed in pg/mL	% Recovery
1	18.25	88.55	73.12	83%
		126.40	132.78	105%
		229.14	186.50	81%
2	27.50	97.33	82.08	84%
		134.94	135.26	100%
		237.00	205.05	87%

Specificity

The Bovine GDF-15 ELISA assay detects low levels of synthetic dimers of human GDF-15 homozygote for wild type (HH), heterozygote HD and the homozygous mutation (DD). Neurturin, GDNF (Glial Cell Line-derived Neurotrophic factor), and recombinant mouse GDF-15 were not detected in the assay.

Cross-Reactant	Expected GDF-15 Concentration (pg/mL)	Observed GDF-15 Concentration (pg/mL)	% Recovery	% Average Recovery
HH/Wild Type	500	38.8	7.8	8.4
	50	4.5	9.0	
HD, DH, HH and DD/ Hetero dimer	500	26.8	5.4	6.1
	50	3.4	6.8	
DD/H202D variant homozygous variant	500	30.2	6.0	6.3
	50	3.3	6.6	
GDNF (Glial Cell Line-derived Neurotrophic factor)	1000	ND	ND	ND
Neurturin	1000	ND	ND	ND
Recombinant Mouse GDF-15	500	ND	ND	ND

ND: Not detectable

Species Immunoreactivity

The antibody pair used in the assay detects Goat, Bovine, Canine, Equine, Feline, Ovine, Porcine, Mouse, Squirrel Monkey and Vervet Monkey samples and it does not detect Rabbit, and Rat samples as represented in table below.

Sample#	Species	Type	O.D.	Conc. (pg/mL)
1	Rabbit	Serum	0.016	ND
2	Rabbit	Serum	0.025	ND
3	Rabbit	Serum	0.015	ND
4	Rabbit	Serum	0.036	ND
5	Rabbit	Serum	0.019	ND
6	Goat	Serum	1.057	104.26
7	Goat	Serum	1.027	100.79
8	Goat	Serum	0.529	47.46
9	Goat	Serum	1.336	138.20
10	Goat	Serum	1.371	142.68
11	Goat	Serum	0.542	48.77
12	Bovine	Serum	0.755	70.74
13	Bovine	Serum	0.206	15.91
14	Bovine	Serum	0.460	40.61
15	Bovine	Serum	0.488	43.38
16	Bovine	Serum	0.550	49.57
17	Bovine	Serum	0.203	15.61
18	Canine	Tissue Extract	0.049	ND
19	Canine	Tissue Extract	0.053	<2.286
20	Canine	Serum	0.670	61.82
21	Canine	Serum	0.135	8.88
22	Canine	Serum	0.186	13.95
23	Canine	Serum	1.997	232.91
24	Equine	Cyst Fluid	0.057 (1:50)	<114.3
25	Equine	Serum	0.113	6.63
26	Equine	Serum	0.091	4.30
27	Equine	Serum	0.099	5.16
28	Equine	Serum	0.080	3.09
29	Equine	Serum	0.072	<2.286
30	Feline	Serum	0.469	41.49
31	Feline	Serum	0.408	35.50
32	Feline	Serum	0.143	9.69
33	Ovine	Serum	0.090	4.19
34	Ovine	Serum	0.149	10.29
35	Porcine	Serum	0.255	20.66
36	Porcine	Serum	0.124	7.76
37	Porcine	Serum	0.170	12.38
38	Mouse	Serum	0.182	13.56
39	Mouse	Serum	0.048 (1:10)	ND
40	Mouse	Serum	0.049 (1:10)	ND
41	Mouse	Serum	0.049 (1:10)	ND
42	Rat	Serum	0.049 (1:10)	ND
43	Rat	Serum	0.050 (1:10)	ND
44	Rat	Serum	0.048 (1:10)	ND
45	Squirrel Monkey	Serum	0.189	14.24
46	Squirrel Monkey	Serum	0.186	13.95
47	Vervet Monkey	Serum	3.206	486.10

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 was within ± 10% of the control as represented in the following table.

Interferent	Interferent Dose	Sample Bovine GDF-15 (pg/mL)	Dosed Sample Bovine GDF-15 (pg/mL)	% Difference
Hemoglobin	1 mg/mL	192.10	191.0	-0.6
	0.5 mg/mL	201.95	199.77	-1.1
Hemoglobin	1 mg/mL	120.61	116.13	-3.7
	0.5 mg/mL	125.71	121.14	-3.6
Biotin	1200 ng/mL	193.24	197.32	2.1
	600 ng/mL	206.57	211.48	2.4
Biotin	1200 ng/mL	120.32	115.95	-3.6
	600 ng/mL	124.64	124.76	0.1
Intralipids	20 mg/mL	198.79	203.56	2.4
	10 mg/mL	203.87	211.04	3.5
Intralipids	20 mg/mL	121.72	123.79	1.7
	10 mg/mL	124.72	133.25	6.8
Bilirubin	0.66 mg/mL	149.18	142.63	-4.4
	0.2 mg/mL	195.75	195.88	0.1
Bilirubin	0.66 mg/mL	90.79	87.82	-3.3
	0.2 mg/mL	121.25	122.88	1.3

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This assay is intended for research use only.

Manufactured by:
Ansh Labs
445 Medical Center Blvd.
Webster, TX 77598-4217 U.S.A.