

# GLP-2 ELISA

# RUO

## AL-174

### INTENDED USE

The Glucagon-like peptide-2 (GLP-2) enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of GLP-2 in EDTA plasma and other biological fluids. This kit is intended for in vitro research use only and is not for use in diagnostic or therapeutic procedures.

### SUMMARY AND EXPLANATION

Glucagon-Like Peptide-2 (GLP-2) is a 33-amino acid gut hormone produced by the intestinal L-cells and various neurons in the central nervous system<sup>1-3</sup>. Posttranslational proteolytic cleavage of proglucagon molecule produces GLP-2 along with GLP-1. Intestinal GLP-2 is co-secreted along with GLP-1 upon nutrient ingestion. GLP-2 is released in response to stimulation by luminal nutrients, such as glucose, fatty acids and dietary fiber<sup>4</sup>. GLP-2 is cleaved by proteolytic enzymes into active form (1-33) and inactive form (3-33). The GLP-2 sequence is highly conserved among mammals and is involved in regulating gut mucosal growth and integrity. The main biological actions of GLP-2 are related to the regulation of energy absorption and maintenance of mucosal morphology, function and integrity of the intestine; however, recent experimental animal studies suggested that GLP-2 exerts beneficial effects on glucose metabolism in conditions related to increased uptake of energy, such as obesity. GLP-2 acts in an endocrine fashion to link intestinal growth and metabolism with nutrient intake<sup>4</sup>. GLP-2 acts as a beneficial factor for glucose metabolism in mice with high-fat diet-induced obesity<sup>5</sup>. GLP-2 and related analogs may be used as therapeutics for short bowel syndrome, Crohn's disease, osteoporosis and as adjuvant therapy during cancer chemotherapy. Measuring plasma levels of GLP-2 in research, preclinical and clinical studies of Type 2 Diabetes and obesity will open new avenues for diagnostics and therapeutics.

### PRINCIPLE OF THE TEST

The GLP-2 is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and Unknown samples are added to GLP-2 antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated GLP-2 antibody. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the third incubation and washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. In principle, the 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 GLP-2 in the samples and calibrators.

### MATERIALS SUPPLIED

#### CAL-174A GLP-2 Calibrator A/Sample Diluent

One bottle, 3 mL, labeled GLP-2 Cal. A/ Sample Diluent, containing 0 ng/mL GLP-2 in protein-based buffer and ProClin 300. Store unopened at 2-8°C until the expiration date.

#### CAL-174B – CAL-174F GLP-2 Calibrators B-F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 0.1 – 7.5 ng/mL GLP-2 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 in plastic vials immediately for multiple use and discard after the run. Avoid repeated freeze thaws.

*Note: The GLP-2 concentration in the GLP-2 calibrators is traceable to the manufacturer's working calibrators. The calibrators are traceable to Teaduglutide (1.02X), a commercial formulation of GLP-2 (1-34aa). Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.*

#### CTR-174-I & CTR-174-II GLP-2 Controls I & II

Two vials, labeled Levels I and II containing low and high GLP-2 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 immediately in plastic vials for multiple use and discard after running. Avoid repeated freeze thaws.

#### PLT-174 Anti-GLP-2 Coated Microtitration strips

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

#### ASB-174 Proglucagon Assay Buffer

One bottle, 8 mL, containing a buffer solution with a non-mercury preservative. Store at 2-8°C until expiration date.

#### BCC-174 GLP-2 Biotin Conjugate Concentrate (50X)

One vial, 0.4 mL, containing detection antibody biotin in a protein-based buffer with a non-mercury preservative. Dilute prior to use in CND-172. Store at 2-8°C until expiration date.

#### CND-172 GLP Conjugate Diluent

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

#### SAR-174 GLP-2 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 28°C until expiration date.

**TMB-100 TMB Chromogen Solution**

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

**STP-100 Stopping Solution**

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°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.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microtitration plate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microplate shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250  $\mu$ L.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.

**WARNINGS AND PRECAUTIONS**

*For in vitro research use only. Not for use in diagnostic procedures.* 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 animal source material (e.g. BSA) or materials used in conjunction with animal 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," 6<sup>th</sup> Edition, 2020.<sup>6</sup>

**WARNING: Potential Chemical Hazard**

Some reagents in this kit contain ProClin 300 and Sodium azide<sup>7</sup> 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**

- a) K<sub>2</sub>EDTA Plasma 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.

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

**PROCEDURAL NOTES**

1. A thorough understanding of this package insert is necessary for successful use of the GLP-2 assay. It is the customer's responsibility 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 various 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 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. **GLP-2 Calibrators B-F and GLP-2 Controls I & II:** Tap and reconstitute GLP-2 Calibrator B-F and GLP-2 Controls I & II each with **1 mL** deionized water. Solubilize, mix well, and use after reconstitution. To homogenize leave for 15 min before use.
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 from moisture.
4. **GLP-2 Antibody-Biotin Conjugate Solution:** The GLP-2 Antibody-Biotin Conjugate Concentrate should be diluted at a ratio of 1-part conjugate to 50 parts of CND-172, according to the number of wells used. If an entire plate is to be used pipet exactly 220  $\mu$ L of the Concentrate in to 11 mL of the diluent.

**ASSAY PROCEDURE**

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

1. Label the microtitration strips to be used.
2. Pipette **50  $\mu$ L** of the Calibrators (Cal A-F), Controls and Unknowns to the appropriate wells.
3. Add **50  $\mu$ L** of the Proglucagon Assay Buffer to each well using a repeater pipette.
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 $\pm$ 2°C).
5. During the last **20-30 minutes** of incubation, prepare the GLP-2 Antibody-Biotin Conjugate Solution by diluting the GLP-2 Biotin Conjugate Concentrate in CND-172 (GLP Conjugate Diluent) as described under the Preparation of the Reagents section of this package insert.
6. Aspirate and wash each strip **5 times** with Wash Solution using an automatic microplate washer.

- Add **100 µL** of the Antibody-Biotin Conjugate Solution to each well using a repeater pipette.
- Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23±2°C).
- Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
- Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
- Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23±2°C).
- Aspirate and wash each strip **5 times** with the Wash Solution using an automatic microplate washer.
- Add **100 µL** of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
- Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-10 min** at room temperature (23±2°C). NOTE: Visually monitor the color development to optimize the incubation time.
- Add **100 µL** of the stopping solution to each well using a precision 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 GLP-2 concentration on X-axis using a cubic regression curve-fit. Alternatively, linear regression curve-fit can be used. Other data reduction methods may give slightly different results.

- Calculate the mean optical density (OD) for each calibrator, control, or unknown.
- Optimum results can be obtained at incubation temperature of (23 ± 2°C).
- Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the GLP-2 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
- Determine the GLP-2 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding GLP-2 concentrations.
- Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 ng/mL (Cal. A / Sample Diluent) and re-assayed.
- Any sample reading lower than the analytical sensitivity should be reported as such.
- Multiply the value by a dilution factor, if applicable.

## LIMITATIONS

The reagents supplied in this kit are optimized to measure GLP-2 levels in human **EDTA plasma**. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.<sup>8</sup>

## QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- GLP-2 ELISA controls or other commercial controls should fall within established confidence limits.

- The confidence limits for GLP-2 controls are printed on the **Calibration Card**.
- A full calibration curve, low and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

## REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean Absorbance	Conc. (ng/mL)
A1, A2	A	0.031 (Blank)	0
B1, B2	B	0.078	0.057
C1, C2	C	0.205	0.14
D1, D2	D	0.64	0.38
E1, E2	E	1.832	1.04
F1, F2	F	3.711	2.6

**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 ng/mL.

### Analytical Sensitivity:

The analytical sensitivity in the GLP-2 ELISA assay, as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 ng/mL) and calibrator B (0.036 ng/mL), is 0.003 ng/mL.

### Linearity:

Human EDTA plasma samples and calibrator F containing various GLP-2 levels were diluted with Calibrator A/sample diluent. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
Sample-1	Neat	0.41		
	1:2	0.20	0.23	111%
	1:4	0.10	0.11	108%
	1:8	0.05	0.05	100%
Sample-2	Neat	1.15		
	1:2	0.57	0.51	89%
	1:4	0.29	0.24	85%
Calibrator F	Neat	1.95		
	1:2	0.98	1.05	107%
	1:4	0.49	0.49	100%
	1:8	0.24	0.24	98%
	1:16	0.12	0.13	102%

Teduglutide was diluted in Calibrator A/sample diluent, and in mouse serum matrices. The % recovery in individual matrices is represented in the following table.

Sample ID	Dilution Factor	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
Teduglutide in Calibrator A	Neat	2.94		
	1:2	1.47	1.50	102%
	1:4	0.74	0.71	96%
	1:8	0.37	0.36	97%
	1:16	0.18	0.18	96%
	1:32	0.09	0.09	95%
Teduglutide in Mouse Serum	Neat	2.94		
	1:2	1.47	1.42	97%
	1:4	0.74	0.69	94%
	1:8	0.37	0.37	101%
	1:16	0.18	0.20	110%
	1:32	0.09	0.10	112%

**Recovery:**

Known amounts of GLP-2 were added to five serum samples containing different levels of endogenous GLP-2. The concentration of GLP-2 was determined before and after the addition of endogenous GLP-2 and the percent recovery was calculated.

Sample ID	Endogenous Value in ng/mL	Expected in ng/mL	Observed in ng/mL	%Recovery
S1	2.62	0.90	0.90	101%
		0.99	0.96	97%
		1.07	1.03	96%
S2	1.85	0.71	0.71	100%
		0.81	0.82	100%
		0.91	0.87	96%
S3	1.74	0.69	0.66	96%
		0.79	0.75	94%
		0.89	0.82	92%
S4	2.27	0.58	0.58	100%
		0.58	0.54	93%
		0.58	0.57	100%
S5	1.52	0.41	0.40	99%
		0.41	0.40	98%
		0.41	0.40	98%

**Hook Effect:**

There is no high-dose hook effect at GLP-2 concentrations up to 78.0 ng/mL.

**Imprecision:**

Reproducibility of the GLP-2 assay was determined in a study using two kit controls. The study included a total of twenty assays, replicates of six per assay (n=120). Representative data were calculated and are presented in the following table.

Sample	Mean Conc.	Within Run		Between Run		Total	
	(ng/mL)	SD	%CV	SD	%CV	SD	%CV
Control I	0.23	0.014	6.14%	0.012	5.16%	0.018	8.02%
Control II	0.83	0.027	3.20%	0.036	4.28%	0.044	5.34%

**Interference:**

When potential interferents (Hemoglobin, biotin, bilirubin, and intralipids) were added at least two times their physiological concentration to control sample, GLP-2 concentrations were within  $\pm 13\%$  of the control as represented in the following table. This study was based on NCCLS EP-7.

Interferent	Interferent Dose	Sample GLP-2 (ng/mL)	Dosed Sample GLP-2 (ng/mL)	% Difference
Hemoglobin	1 mg/mL	0.67	0.74	11.1
	0.5 mg/mL	0.75	0.77	2.7
	0.1 mg/mL	0.80	0.80	-0.1
Hemoglobin	1 mg/mL	0.25	0.26	1.2
	0.5 mg/mL	0.24	0.26	8.1
	0.1 mg/mL	0.27	0.26	-1.8
Biotin	1200 ng/mL	0.72	0.73	2.0
	600 ng/mL	0.76	0.83	8.8
	200 ng/mL	0.70	0.79	12.2
Biotin	1200 ng/mL	0.24	0.24	0.0
	600 ng/mL	0.26	0.26	0.8
	200 ng/mL	0.26	0.26	-0.9
Intralipids	20 mg/mL	0.73	0.76	5.1
	10 mg/mL	0.76	0.73	-2.9
	5 mg/mL	0.79	0.84	5.7
Intralipids	20 mg/mL	0.25	0.27	5.5
	10 mg/mL	0.26	0.26	-0.7
	5 mg/mL	0.26	0.28	6.4
Bilirubin	0.66 mg/mL	0.51	0.53	4.0
	0.2 mg/mL	0.67	0.71	5.5
Bilirubin	0.66 mg/mL	0.19	0.20	2.4
	0.2 mg/mL	0.25	0.25	0.8

**Analytical Specificity:**

The GLP-2 assay detects 1-34 and 3-34 aa sequence of GLP-2 and does not detect 1-18 aa, 3-18aa and 19-34aa peptides of GLP-2. The cross-reactivity to other closely related analytes is listed below.

Cross-Reactant	Concentration (ng/mL)	% Cross-reactivity
Oxyntomodulin	100	ND
Glucagon (1-29)	100	ND
GLP-1 (1-36)	100	ND
GLP-1 (7-36)	100	ND
GLP-1 (9-36)	100	ND
GLP-2 (1-34)	15	24%
GRPP	100	ND
MPGF-1	10	2.4%
MPGF-2	10	ND
Insulin	10	ND
C-Peptide	10	ND
Thyroglobulin	10	ND
Glicentin	100	ND
Teduglutide	1.47	102%
GLP-2 (1-34)	11.3	26%
GLP-2 (3-34)	1.1	186%
GLP-2 (1-18)	110	ND
GLP-2 (19-34)	110	ND
GLP-2 (3-18)	110	ND

ND= Not Detectable

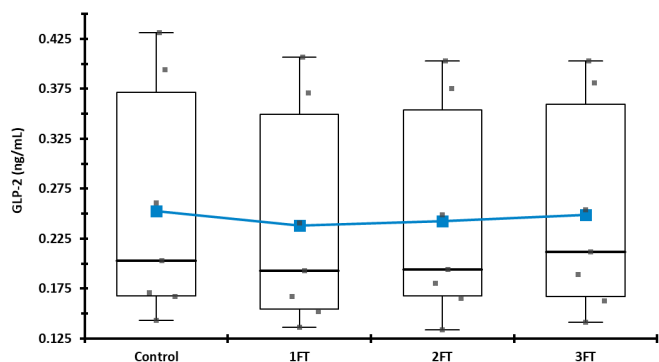
**Species Immunoreactivity:**

The antibody pair used in GLP-2 ELISA assay detects bovine, canine, equine, goat, ovine, rat, porcine, squirrel monkey and vervet monkey samples and does not detect rabbit and mouse samples as represented below.

Sample#	Species	Type	O.D.	Conc. (ng/mL)
1	Rabbit	Serum	0.01	<0.037
2	Rabbit	Serum	0.00	ND
3	Bovine	Serum	0.21 (1:2)	0.26
4	Bovine	Serum	0.86	0.42
5	Bovine	Serum	0.82	0.40
6	Canine	Serum	0.00	<0.037
7	Canine	Serum	0.09	0.06
8	Canine	Serum	0.07	0.06
9	Mouse	Serum	0.005 (1:10)	<0.37
10	Mouse	Serum	0.005 (1:10)	<0.37
11	Equine	Serum	0.11	0.08
12	Equine	Serum	0.22	0.14
13	Goat	Serum	3.52	1.93
14	Goat	Serum	1.63	0.75
15	Ovine	Serum	0.41	0.23
16	Ovine	Serum	0.15	0.12
17	Ovine	Serum	0.22	1.71
18	Rat	Serum	0.20 (1:10)	1.57
19	Rat	Serum	0.17 (1:10)	1.36
20	Porcine	Serum	0.75	0.52
21	Porcine	Serum	0.96	0.66
22	Squirrel Monkey	Serum	3.91	3.33
23	Squirrel Monkey	Serum	3.94	3.38
24	Vervet Monkey	Serum	3.96	3.40

**Sample Stability:**

Seven K<sub>2</sub>EDTA plasma samples were frozen and thawed three times to evaluate sample stability. Data was analyzed using Analyse-it for Microsoft excel and is plotted below.



0.700	0.28
0.800	0.33
0.900	0.40

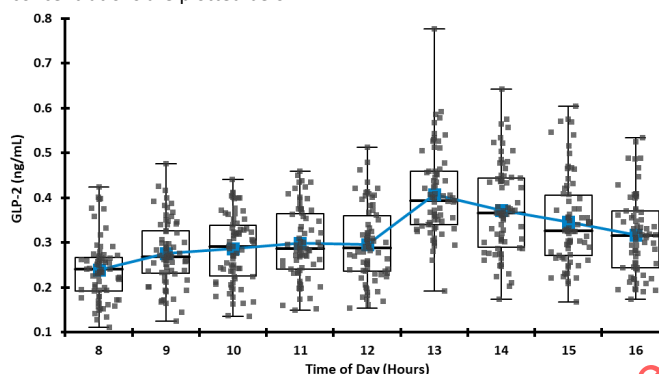
NOTE: It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

**REFERENCES**

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**Mixed-Meal Tolerance Test (MMTT):**

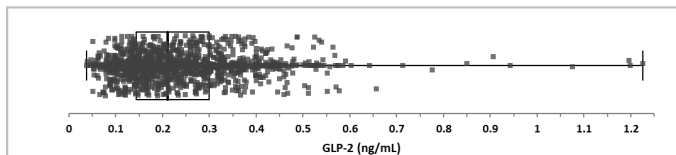
Mixed-Meal Tolerance Test (MMTT) was performed on 71 subjects. Blood was drawn hourly between 8:00 and 16:00 with food intake at 8:00 and 12:00. GLP-2 concentrations are plotted below <sup>9</sup>.



Time Point (Hours)	N	Mean	Median	95% CI
8	71	0.240	0.241	0.223 - 0.256
9	71	0.277	0.269	0.259 - 0.294
10	71	0.286	0.291	0.268 - 0.304
11	71	0.299	0.287	0.280 - 0.317
12	71	0.296	0.289	0.277 - 0.316
13	71	0.408	0.394	0.385 - 0.431
14	71	0.372	0.365	0.348 - 0.396
15	71	0.347	0.326	0.323 - 0.371
16	71	0.317	0.315	0.297 - 0.338

**Expected Values:**

Expected GLP-2 concentration in undifferentiated population (diabetic and non-diabetic) was calculated by evaluating 1534 samples in Ansh Labs GLP-2 ELISA. The frequency distribution was calculated using Analyse-It<sup>®</sup> for Microsoft Excel and is shown below.



n	GLP-2 (ng/mL)			
	Mean	Median	Range	95% CI
1534	0.23	0.21	0.04 - 1.23	0.23 - 0.24
Quantile		GLP-2 (ng/mL)		
0.100		0.10		
0.200		0.13		
0.300		0.16		
0.400		0.18		
0.500		0.21		
0.600		0.24		

Refer to package insert for exact specifications

This assay is intended for in-vitro research use only. Not for use in diagnostic procedures.

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Manufactured by:  
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