**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 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. 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. 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. GLP-2 acts as a beneficial factor for glucose metabolism in mice with high-fat diet-induced obesity. 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 avenue 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 enzyme reaction takes place. The enzyme substrate reaction is detected by colorimetric 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 MPGF in protein based buffer and Pro-Clean 400. 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 Pro-Clean 400. 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. The GLP-2 concentration in the GLP-2 calibrators is traceable to the manufacturer’s working calibrators. 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 Pro-Clean 400. 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-105**
  - 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. Microtiter 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 µL.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.

WARNINGS AND PRECAUTIONS
For Research Use Only. Not for use in diagnostic procedures.
The following precautions should be observed:
1. Follow good laboratory practice.
2. Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.
3. 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," 5th Edition, 2007. 5

WARNING: Potential Chemical Hazard
Some reagents in this kit contain Pro-Clean 400 and Sodium azide as a preservative. Pro-Clean 400 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) KEDTA 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.

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 µL of the Calibrators (Cal A-F), Controls and Unknowns to the appropriate wells.
3. Add 50 µL 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±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.

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 Biotin Conjugate 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 µL of the Concentrate in to 11 mL of the diluent.

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6. Aspirate and wash each strip 5 times with Wash Solution using an automatic microplate washer.
7. Add 100 µL of the Antibody-Biotin Conjugate Solution to each well using a repeater pipette.
8. 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).
9. Aspirate and wash each strip 5 times with the Wash Solution using an automatic microplate washer.
10. Add 100 µL of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
11. 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).
12. Aspirate and wash each strip 5 times with the Wash Solution using an automatic microplate washer.
13. Add 100 µL of the TMB chromogen solution to each well using a precision pipette. Avoid exposure to direct sunlight.
14. 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.
15. 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.

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 GLP-2 concentrations in ng/mL along the x-axis, using a cubic regression curve-fit.
4. 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.
5. Any sample reading higher than the highest calibrator should be appropriately diluted with the 0 ng/mL (Cal A / Sample Diluent) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. 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.

### 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

<table>
<thead>
<tr>
<th>Well Number</th>
<th>Well Contents</th>
<th>Mean Absorbance</th>
<th>Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrators</td>
<td></td>
<td>0.029 (Blank)</td>
<td>0</td>
</tr>
<tr>
<td>A1, A2</td>
<td>A</td>
<td>0.074</td>
<td>0.14</td>
</tr>
<tr>
<td>B1, B2</td>
<td>B</td>
<td>0.194</td>
<td>0.32</td>
</tr>
<tr>
<td>C1, C2</td>
<td>C</td>
<td>0.589</td>
<td>1</td>
</tr>
<tr>
<td>D1, D2</td>
<td>D</td>
<td>1.760</td>
<td>3</td>
</tr>
<tr>
<td>E1, E2</td>
<td>E</td>
<td>3.570</td>
<td>7.5</td>
</tr>
</tbody>
</table>

**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 (0.14 ng/mL), is 0.011 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.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Dilution Factor</th>
<th>Expected Conc. (ng/mL)</th>
<th>Observed Conc. (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample-1</td>
<td>Neat</td>
<td>1.571</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>0.786</td>
<td>0.874</td>
<td>111%</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>0.393</td>
<td>0.423</td>
<td>108%</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>0.196</td>
<td>0.197</td>
<td>100%</td>
</tr>
<tr>
<td>Sample-2</td>
<td>Neat</td>
<td>4.406</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>2.203</td>
<td>1.962</td>
<td>89%</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>1.102</td>
<td>0.940</td>
<td>85%</td>
</tr>
<tr>
<td>Calibrator F</td>
<td>Neat</td>
<td>7.500</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>3.750</td>
<td>4.022</td>
<td>107%</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>1.875</td>
<td>1.880</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>0.938</td>
<td>0.922</td>
<td>98%</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>0.469</td>
<td>0.479</td>
<td>102%</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Conc. (ng/mL)</th>
<th>SD</th>
<th>%CV</th>
<th>Within Run</th>
<th>SD</th>
<th>%CV</th>
<th>Between Run</th>
<th>SD</th>
<th>%CV</th>
<th>Total</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.882</td>
<td>0.054</td>
<td>6.09%</td>
<td>0.046</td>
<td>5.19%</td>
<td>0.071</td>
<td>8.00%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.200</td>
<td>0.102</td>
<td>3.20%</td>
<td>0.137</td>
<td>4.28%</td>
<td>0.171</td>
<td>5.34%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Analytical Specificity:
The GLP-2 sequence is highly conserved among mammals. Antibody pair used in the assay detects human GLP-2 and cross-reactivity to other closely related analytes is listed below.

<table>
<thead>
<tr>
<th>Cross-Reactant</th>
<th>Concentration</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxyntomodulin</td>
<td>100 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>Glucagon (1–29)</td>
<td>100 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>GLP-1 (1–36)</td>
<td>100 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>GLP-1 (7-36 amide)</td>
<td>100 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>GLP-1 (9-36 amide)</td>
<td>100 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>GLP-2 (1–34)</td>
<td>7.5 ng/mL</td>
<td>100%</td>
</tr>
<tr>
<td>GRPP</td>
<td>100 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>MPGF</td>
<td>10 ng/mL</td>
<td>6.0%</td>
</tr>
<tr>
<td>Insulin</td>
<td>10 ng/mL</td>
<td>ND</td>
</tr>
<tr>
<td>C-peptide</td>
<td>10 ng/mL</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND=Non-Detectable

REFERENCES


For Illustrative Purposes Only Refer to package insert included with the product for exact specifications.