GLP-1 (Active 7-36) ELISA

For the quantitative determination of active glucagon-like peptide-1 (7-36) levels in plasma

For Research use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 43-GP1HU-E01
Size: 96 Wells
Version: V10/RUO/2018-03 ALPCO – March 22, 2018
INTENDED USE
This highly sensitive ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of bioactive glucagon-like peptide-1 (7-36) [GLP-1 (7-36)] level in plasma samples. The primary amino acid sequence of GLP-1 peptide is identical among mammalian species, i.e. rat, mouse, pig, human, etc. This kit is for research purposes only.

ASSAY PRINCIPLE
This ELISA is designed, developed and produced for the quantitative measurement of bioactive GLP-1 (7-36) in plasma samples. The assay utilizes the two-site “sandwich” technique with two selected GLP-1 (7-36) specific antibodies.

Assay standards, controls and test samples are directly added to wells of a microplate that is coated with streptavidin. Subsequently, a mixture of biotinylated GLP-1 (7-36) specific antibody and a horseradish peroxidase (HRP) conjugated GLP-1 (7-36) specific antibody is added to each well. After the first incubation period, a “sandwich” immunocomplex of “Streptavidin – Biotin-Antibody – GLP-1(7-36) – HRP-conjugated antibody” is formed and attached to the wall of the plate. The unbound HRP-conjugated antibody is removed in a subsequent washing step. For the detection of this immunocomplex, each well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to GLP-1 (7-36) on the wall of the microtiter well is directly proportional to the amount of GLP-1 (7-36) in the sample.

REAGENTS: Preparation and Storage
This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. Streptavidin Coated Microplate (Cat. No. 10040B)
One well-breakable microplate with 12 x eight strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiry date on the kit box.

2. GLP-1 Tracer Antibody (Cat. No. 30229)
One vial containing 0.6 mL HRP-labeled Anti-GLP-1 specific antibody in a stabilized protein matrix. This reagent must be mixed with GLP-1 (7-36) Capture Antibody and the tracer antibody diluent before use (for details see Assay Procedure). This reagent should be stored at 2 – 8°C and is stable until the expiry date on the kit box.

3. GLP-1 (7-36) Capture Antibody (Cat. No. 30230)
One vial containing 0.6 mL of biotinylated GLP-1 (7-36) specific antibody. It should be used only after being mixed with GLP-1 Tracer Antibody and the tracer antibody diluent according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiry date on the kit box.

4. Wash Concentrate (Cat. No. 10010)
One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide and non-mercury based
preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiry date on the kit box.

5. HRP Substrate (Cat. No. 10020)
One bottle contains 24 mL of tetramethylbenzidine (TMB) with stabilized hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. Stop Solution (Cat. No. 30357)
One bottle contains 12 mL of sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiry date on the kit box.

7. GLP-1 Standards (Cat. No. 30441 – 30446)
Six vials containing different levels of lyophilized GLP-1 (7-36) in a liquid protein matrix with a non-azide, non-mercury based preservative. Refer to vials for exact concentration for each standard. These reagents should be stored at 2 – 8°C and are stable until the expiry date on the kit box.

8. GLP-1 Controls (Cat. No. 30447 – 30448)
Two vials containing different levels of lyophilized GLP-1 (7-36) in a liquid protein matrix with a non-azide, non-mercury based preservative. Refer to vials for exact concentration range for each control. Both controls should be stored at 2 – 8°C and are stable until the expiry date on the label.

9. Tracer Antibody Diluent (Cat. No. 30017)
One vial containing 12 mL ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiry date on the kit box.

SAFETY PRECAUTIONS
The reagents must be used in a professional laboratory environment and are for research use only. Source material (e.g. highly purified bovine serum albumin) of bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, and 1000 µL etc.
2. Repeating dispenser suitable for delivering 100 µL
3. Disposable pipette tips suitable for above volume dispensing
4. Disposable 12 x 75 mm or 13 x 100 glass/plastic tubes
5. Disposable plastic 100 mL and 1000 mL bottle with caps
6. Aluminum foil
7. Deionized or distilled water
8. Vortex
9. Plastic microtiter well cover or polyethylene film
10. ELISA plate shaker
11. ELISA multichannel wash bottle or automatic (semi-automatic) washing system
12. Spectrophotometric microplate reader capable of reading absorbance at 450 nm
13. DPP-4 Inhibitor
14. BD™ P700 Blood Collection and Preservation System (contains a DPP-4 protease inhibitor cocktail) or lavender top Vacutainer® EDTA-plasma tubes (see Sample Collection below)
15. Ice Bath
16. Timer

SAMPLE COLLECTION

(1) No special preparation of individuals is necessary prior to sample collection. However, fasting samples and non-fasting/glucose induced samples may significantly affect bioactive GLP-1 (7-36) levels.
(2) Samples should not be taken from donors taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to sample collection.
(3) BD™ P700 Blood Collection and Preservation System (contains a DPP-4 protease inhibitor cocktail) must be used for sample collection, if a direct Active GLP-1 (7-36) measurement will be performed by using this ELISA kit.
(4) As an alternative to BD™ P-700 tubes, whole blood should be collected into a lavender top Vacutainer EDTA-plasma tube. It is very important to immediately add the appropriate amount of DPP-4 inhibitor to the collected EDTA whole blood immediately after the collection (within 30 seconds). Refer to DPP-4 inhibitor manufacturer’s instruction. Invert tube to mix well and place the tube on ice bath. Centrifuge the tube at 1000 x g for 10 minutes in a refrigerated centrifuge. A solid phase extraction procedure should be used for this type of sample before performing the GLP-1 assay.
(5) Plasma samples should be stored at 2 – 8°C if they will be tested within 3 hours of collection. For longer storage, it is recommended to store the plasma sample at -70°C. Aliquot samples before freezing if necessary.

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) Wash Concentrate (Cat. 10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
(3) Reconstitute all standards (Cat. 30441-30446) and controls (Cat. 30447-30448) by adding 1.0 mL of demineralized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by gentle vortexing. These reconstituted standards and controls must be stored at -20°C or below. Do not exceed 3 freeze-thaw cycles.

2. Test Sample Preparation

(1) For direct measuring Active GLP-1 (7-36), BD™ P-700 Blood Collection and Preservation System must be used for sample collection. There is not any sample preparation before the assay. (See sample collection for alternative to BD™ P-700 tubes.)
(2) It is optional to perform a solid-phase sample extraction procedure for all test samples that are collected with DPP-IV inhibitor cocktail other than BD™ P-700 tube. If a pre-assay sample extraction procedure is it is recommended to use a solid phase sample extraction procedure with a GLP-1 Sample Extraction Kit.
3. Assay Procedure

(1) Place a sufficient number of streptavidin-coated microwell strips/wells (Cat. 10040B) in a holder to run GLP-1 (7-36) standards, controls and unknown samples in duplicate.

(2) Test Configuration:

<table>
<thead>
<tr>
<th>ROW</th>
<th>STRIP 1</th>
<th>STRIP 2</th>
<th>STRIP 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>STD 1</td>
<td>STD 5</td>
<td>SAMPLE 1</td>
</tr>
<tr>
<td>B</td>
<td>STD 1</td>
<td>STD 5</td>
<td>SAMPLE 1</td>
</tr>
<tr>
<td>C</td>
<td>STD 2</td>
<td>STD 6</td>
<td>SAMPLE 2</td>
</tr>
<tr>
<td>D</td>
<td>STD 2</td>
<td>STD 6</td>
<td>SAMPLE 2</td>
</tr>
<tr>
<td>E</td>
<td>STD 3</td>
<td>C 1</td>
<td>SAMPLE 3</td>
</tr>
<tr>
<td>F</td>
<td>STD 3</td>
<td>C 1</td>
<td>SAMPLE 3</td>
</tr>
<tr>
<td>G</td>
<td>STD 4</td>
<td>C 2</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>STD 4</td>
<td>C 2</td>
<td></td>
</tr>
</tbody>
</table>

(3) Prepare GLP-1 (7-36) Antibody Mixture: mixing GLP-1 Tracer Antibody and Capture Antibody by 1:21 fold dilution of the Tracer Antibody (Cat. 30229) and by 1:21 fold dilution of the biotinylated Capture Antibody (Cat. 30230) with the Tracer Antibody Diluent. For each strip, it is required to mix 1 mL of the Tracer Antibody Diluent (Cat. 30017) with 50 µL the Capture Antibody and 50 µL of the Tracer Antibody in a clean test tube.

(4) Add 100 µL of standards, controls and test samples into the designated microwell.

(5) Add 100 µL of GLP-1 (7-36) Antibody Mixture to each well.

(6) Cover the plate with one plate sealer and incubate plate at 2-8°C, static for 20 - 24 hours.

(7) Remove plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(8) Add 200 µL of ELISA HRP Substrate (Cat. 10020) into each of the wells.

(9) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.

(10) Incubate plate at room temperature, static for 20 min.

(11) Remove the aluminum foil and plate sealer. Add 50 µL of ELISA Stop Solution (Cat. 30357) into each of the wells. Mix gently.

(12) Read the absorbance at wavelength 450 nm/620 nm or 450 nm/650 nm within 10 minutes in a microplate reader.

PROCEDURAL NOTES

1. Failure to collect samples as above may return erroneous results due to endogenous DPP-4 activity.
2. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
3. For samples with concentration higher than level 6 standard, it is recommended to dilute the sample with an appropriate GLP-1 free buffer matrix (e.g. standard zero) for a more accurate result.
4. Keep light-sensitive reagents in the original amber bottles.
5. Store any unused streptavidin-coated strips in the foil zipper bag with desiccant to protect from moisture.
6. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
7. Incubation times or temperatures other than those stated in this insert may affect the results.
8. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher %CV of duplicate readings.
9. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

**INTERPRETATION OF RESULTS**
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the average absorbance of all standard levels on the ordinate against the standard concentrations on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may be used for the calculation of results. Point-to-Point or Quadratic curve fit is recommended.

The GLP-1 (7-36) concentrations for the controls and test samples are read directly from the standard curve using their respective corrected absorbance.

**EXAMPLE DATA AND STANDARD CURVE**
A typical absorbance data and the resulting standard curve from this GLP-1 ELISA are represented. The example curve was generated using a point-to-point curve fit with linear axes. Other curve fits using linear or logarithmic axes may also be used.

**This example curve should not be used in lieu of standard curve run with each assay:**

<table>
<thead>
<tr>
<th>Well I.D.</th>
<th>OD 450 nm/650 nm Absorbance Readings</th>
<th>Average</th>
<th>Corrected Results (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 pmol/mL</td>
<td>0.010 0.011</td>
<td>0.011</td>
<td>0.000</td>
</tr>
<tr>
<td>0.64 pmol/L</td>
<td>0.054 0.057</td>
<td>0.055</td>
<td>0.044</td>
</tr>
<tr>
<td>2.20 pmol/L</td>
<td>0.157 0.174</td>
<td>0.165</td>
<td>0.154</td>
</tr>
<tr>
<td>6.20 pmol/L</td>
<td>0.451 0.451</td>
<td>0.451</td>
<td>0.440</td>
</tr>
<tr>
<td>21.00 pmol/L</td>
<td>1.399 1.370</td>
<td>1.385</td>
<td>1.374</td>
</tr>
<tr>
<td>48.00 pmol/L</td>
<td>2.741 2.785</td>
<td>2.763</td>
<td>2.752</td>
</tr>
<tr>
<td>Control I</td>
<td>0.456 0.458</td>
<td>0.457</td>
<td>0.446 6.29</td>
</tr>
<tr>
<td>Control III</td>
<td>0.918 0.932</td>
<td>0.925</td>
<td>0.914 13.71</td>
</tr>
</tbody>
</table>
LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration or international standard available for GLP-1 (7-36) measurement, the values of assay standards were established using a highly purified GLP-1 (7-36) peptide and validated by the manufacturer. Results obtained with different assay methods or kits cannot be used interchangeably.
2. For unknown sample values read directly from the assay that are greater than standard level-6, it is recommended to measure a diluted sample for more accurate measurement.
3. Bacterial or fungal contamination of plasma samples or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL
To assure the validity of the results each assay should include adequate controls with known GLP-1 (7-36) levels.

PERFORMANCE CHARACTERISTICS
Sensitivity
The sensitivity of this highly sensitive Active GLP-1 (7-36) ELISA is determined by 3 times the standard deviation above zero standard on 12 replicate determinations is approximately 0.05 pmol/L.

Specificity
This Bioactive GLP-1 (7-36) assay is a specific measure of GLP-1 (7-36). It is expected that this assay does not detect following peptides.

GLP-1 (7-36) 100%
GLP-1 (9-36) < 0.1%
GLP-1 (9-37) < 0.1%
GLP-1 (7-37) < 0.1%
GLP-1 (1-36) < 0.1%
GLP-2 < 0.1%
Glucagon < 0.1%
Precision
The intra-assay precision was determined by 8 replicates for two control samples in a single assay. A very satisfactory within assay CV% was obtained as indicated below.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Average GLP-1 (7-36) (n = 8)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>3.49 pmol/L</td>
<td>0.19</td>
<td>5.4%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>10.18 pmol/L</td>
<td>0.26</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

The inter-assay precision was determined by 13 individual assays in different dates with two control samples. A very satisfactory between assay CV% was observed as indicated below.

<table>
<thead>
<tr>
<th>Sample#</th>
<th>Average GLP-1 (7-36) (n = 13)</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>4.13 pmol/L</td>
<td>0.16</td>
<td>3.9%</td>
</tr>
<tr>
<td>Sample 2</td>
<td>2.14 pmol/L</td>
<td>0.68</td>
<td>5.6%</td>
</tr>
</tbody>
</table>

Spike Recovery
Two samples were spiked with each other using the sample volume (200 μL + 200 μL), measured with this assay and the spike recovery calculated.

<table>
<thead>
<tr>
<th>Samples (pmol/L)</th>
<th>Samples (pmol/L)</th>
<th>Measured value (pmol/L)</th>
<th>Expect value (pmol/L)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.45</td>
<td>7.49</td>
<td>18.56</td>
<td>19.98</td>
<td>92.9</td>
</tr>
<tr>
<td>6.67</td>
<td>18.62</td>
<td>11.25</td>
<td>12.65</td>
<td>89.0</td>
</tr>
<tr>
<td>2.40</td>
<td>7.61</td>
<td>4.12</td>
<td>5.00</td>
<td>82.4</td>
</tr>
</tbody>
</table>

Linearity
Two samples were diluted with GLP-1 (7-36) zero standard matrix. These diluted samples were measured in this assay and the linear recovery calculated.

<table>
<thead>
<tr>
<th>Sample Dilution format</th>
<th>GLP-1 (7-36) zero human serum</th>
<th>Measured value (pmol/L)</th>
<th>Expect value (pmol/L)</th>
<th>Linear recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 μL</td>
<td>250 μL</td>
<td>0.64</td>
<td>0.70</td>
<td>91.3</td>
</tr>
<tr>
<td>100 μL</td>
<td>200 μL</td>
<td>1.06</td>
<td>1.39</td>
<td>76.1</td>
</tr>
<tr>
<td>150 μL</td>
<td>150 μL</td>
<td>2.21</td>
<td>2.12</td>
<td>104.3</td>
</tr>
<tr>
<td>200 μL</td>
<td>100 μL</td>
<td>2.88</td>
<td>2.82</td>
<td>102.2</td>
</tr>
<tr>
<td>250 μL</td>
<td>50 μL</td>
<td>4.18</td>
<td>3.55</td>
<td>117.9</td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 μL</td>
<td>250 μL</td>
<td>2.70</td>
<td>2.79</td>
<td>96.7</td>
</tr>
<tr>
<td>100 μL</td>
<td>200 μL</td>
<td>5.09</td>
<td>5.55</td>
<td>91.8</td>
</tr>
<tr>
<td>150 μL</td>
<td>150 μL</td>
<td>7.49</td>
<td>8.34</td>
<td>89.8</td>
</tr>
<tr>
<td>200 μL</td>
<td>100 μL</td>
<td>11.40</td>
<td>11.13</td>
<td>102.5</td>
</tr>
<tr>
<td>250 μL</td>
<td>50 μL</td>
<td>13.37</td>
<td>13.89</td>
<td>96.3</td>
</tr>
</tbody>
</table>
This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. The manufacturer disclaims any implied warranty of merchantability or fitness for a particular purpose, and in no event shall be liable for consequential damages. Replacement of the product or refund of the purchased price is the exclusive remedy for the purchaser. This warranty gives the user specific legal rights and other rights may vary state to state.

REFERENCES

Short Assay Protocol:
- Add 100 μL per well of standards, control and sample
- Add 100 μL of Antibody Mixture
- Incubate 20 - 24 hour at 2-8°C, static
- Wash strips with diluted wash buffer
- Add 200 μL/well of TMB substrate
- Incubate 20 min at RT, static
- Add 50 μL stop solution
- Read strips at OD 450 nm/620 nm or 450 nm/650 nm