GLP-1 (7-36 and 9-36) ELISA

For the quantitative determination of glucagon-like peptide-1 (7-36) and (9-36) levels in plasma

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

Catalog Number:	43-GPTHU-E01
Size:	96 wells
Version:	v1 / US / Jan 2010 - ALPCO 4/14/2010

ALPCO Diagnostics

26G Keewaydin Drive • Salem, NH 03079
Phone: (800) 592-5726 • Fax: (603) 898-6854
www.alpco.com • Email: web@alpco.com

INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of glucagon-like peptide-1 (GLP-1) (7-36) and (9-36) in human plasma samples. The primary amino acid sequence of GLP-1 peptide is identical among mammalian species, i.e., rat, mouse, pig, human, etc. This ELISA has been developed for use with human plasma samples only. ***This kit is for research purposes only. It is not for use in diagnostic procedures.***

ASSAY PRINCIPLE

This ELISA is designed, developed, and produced for the quantitative measurement of GLP-1 (7-36) and (9-36) in plasma samples. The assay utilizes the two-site "sandwich" technique with two selected GLP-1 antibodies. This assay uses the same assay standards and tracer antibodies as the GLP-1 (Active 7-36) ELISA (catalog no. 43-GP1HU-E01).

Assay standards, controls, and test samples are added directly to wells of a microplate that is coated with streptavidin. Subsequently, a mixture of biotinylated GLP-1 specific antibody and a horseradish peroxidate (HRP) conjugated GLP-1 specific antibody is added to each well. After the first incubation period, a "sandwich" immunocomplex of "streptavidin – biotin-antibody – GLP-1(7-36)/(9-36) – HRP conjugated antibody" is formed and attaches to the walls 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)/(9-36) on the walls of the microtiter wells is directly proportional to the amount of GLP-1 (7-36/9-36) in the sample.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiry date of the kit refer to the label on the kit box. All components are stable until this expiry date. This product is manufactured by Epitope Diagnostics, Inc.

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. 10040)**
   One well-breakable microplate with twelve 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 label.
2. **Tracer Antibody (Cat. No. 30360)**
   One vial containing 0.6 ml HRP labeled Anti-GLP-1 specific antibody in a stabilized protein matrix. This reagent must be mixed with the 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 label.
3. **Capture Antibody (Cat. No. 30361)**
   One vial containing 0.6 ml of biotinylated GLP-1 specific antibody. It should be used only after being mixed with the Tracer Antibody and the Tracer Antibody Diluent according to the ASSAY PROCEDURE. 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 20 ml of 30 fold concentrate. Before use the contents must be diluted with 580 ml of distilled or deionized water and mixed well. Upon dilution this yields a working Wash Buffer 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 label.
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 expiry date on the label.
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 label.
7. **Standards (Cat. No. 30261 – 30265)**
   Five 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 vial label for exact concentration for each standard.** These reagents should be stored at 2 – 8°C and are stable until the expiry date on the label.

8. Controls (Cat. No. 30266 – 30267)
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 vial label 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 of ready to use buffer. It should be used only for Tracer Antibody dilution according to the ASSAY PROCEDURE. This reagent should be stored at 2 – 8°C and is stable until the expiry date on the label.

10. Plate Sealers
Three plate sealers.

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 1,000 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 1,000 ml bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. ELISA plate shaker.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
11. BD™ P700 Blood Collection and Preservation System.

OR *(See Sample Collection below.)*

12. Lavender top Vacutainer® EDTA-plasma tubes.
13. DPP-4 Inhibitor.
14. Refrigerated centrifuge (1,000 x g).
15. Ice bath.

SAMPLE COLLECTION

(1) No special preparation of the individual is necessary prior to sample collection. However, fasting samples and non-fasting/glucose induced samples may present great significance for GLP-1 (7-36)/(9-36) levels.
(2) Both the BD™ P700 Blood Collection and Preservation System (contains a DPP-4 protease inhibitor cocktail) and lavender top Vacutainer® EDTA-plasma tubes can be used for sample collection.
(3) If a Vacutainer® EDTA-plasma tube is used for sample collection, it is recommended (but not necessary) to add an appropriate amount of DPP-4 inhibitor to the collected EDTA whole blood right after collection. Refer to DPP-4 inhibitor manufacturer's instructions. Invert tube to mix well and place the tube in an ice bath. Centrifuge the tube at 1,000 x g for 10 minutes in a refrigerated centrifuge.
Note: Since samples with DPP-4 inhibitor are strongly recommended for measurement of GLP-1 (Active 7-36), the DPP-4 inhibitor should be included in the sample collection if the same sample will be used for both GLP-1 (Active 7-36) and GLP-1 (7-36)/(9-36) *measurements.*
(4) 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 samples at -70°C. Prepare aliquots of the 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 Buffer must be diluted to working solution prior to use. Please see REAGENTS section for details.
(3) Reconstitute all standards and controls by adding **1.0 ml** of deminerialized 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

EDTA-plasma samples with or without DPP-IV inhibitor can be directly measured for the GLP-1 (7-36/9-36) concentration.

3. Assay Procedure

(1) Place a sufficient number of streptavidin coated microwell strips/wells in a holder to run standards, controls, and unknown samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 2
B	STD 1	STD 5	SAMPLE 2
C	STD 2	C 1	SAMPLE 3
D	STD 2	C 1	SAMPLE 3
E	STD 3	C 2	SAMPLE 4
F	STD 3	C 2	SAMPLE 4
G	STD 4	SAMPLE 1	
H	STD 4	SAMPLE 1	

(3) Prepare Antibody Mixture: mix Tracer Antibody and Capture Antibody by 1:21 fold dilution of the Tracer Antibody and by 1:21 fold dilution of the biotinylated Capture Antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 1 ml of the Tracer Antibody Diluent (30017) with 50 µL the Capture Antibody (30361) and 50 µL of the Tracer Antibody (30360) in a clean test tube.
(4) Add **100 µL** of standards, controls, and test samples to the designated microwells.
(5) Add **100 µL** of 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 Buffer into each well and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
(8) Add **200 µL** of HRP Substrate to each of the wells.
(9) Cover the plate with one plate sealer and with aluminum foil to avoid exposure to light.
(10) Incubate plate at room temperature, static for **20 minutes**.
(11) Remove the aluminum foil and plate sealer. Add **50 µL** of Stop Solution to each of the wells. Mix gently.
(12) Read the absorbance at wavelength **450nm/620 nm** within 10 minutes in a microplate reader.

PROCEDURAL NOTES

1. 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.
2. If a sample has a value higher than the level 5 standard, it is recommended to dilute the sample with an appropriate GLP-1 free human plasma matrix or an appropriate buffer matrix (e.g., zero standard), and then assay again for a more accurate report.
3. Keep light sensitive reagents in the original amber bottles.
4. Store any unused streptavidin coated strips in the foil zipper bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation times or temperatures other than those stated in this insert may affect the results.
7. Avoid air bubbles in the microwells as this could result in lower binding efficiencies and higher CV% of duplicate readings.
8. 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 zero standard (0 ng/ml) from the average absorbance of all other readings to obtain the corrected absorbances.
3. The standard curve is generated by the corrected absorbances 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 also be used for the calculation of results. It is recommended to use a **Point-to-Point** or **Quadratic** curve fit.

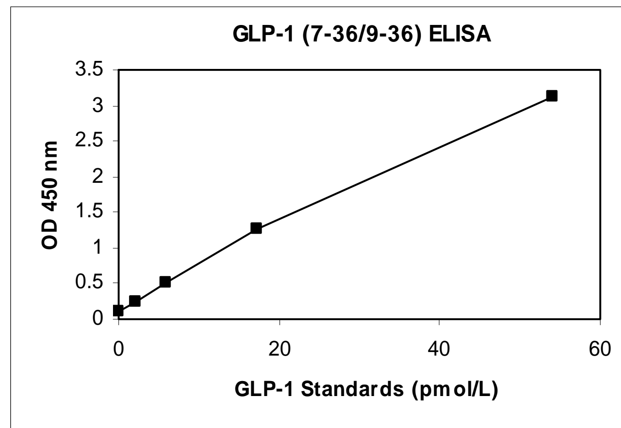
The GLP-1 concentrations for the controls and test samples are read directly from the standard curve using their respective corrected absorbances. If either log-log graph paper or a computer assisted data reduction program utilizing logarithmic transformation is used, samples having corrected absorbances between the 2nd standard and the next highest standard should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2}^{nd}\text{ STD)}} \times \text{Value of the 2}^{nd}\text{ STD}$$

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this GLP-1 (7-36 and 9-36) ELISA are represented. **This curve should not be used in lieu of a standard curve run with each assay.**

Well	OD 450 nm Absorbance			Results pmol/L
	Readings	Average	Corrected	
0 pmol/ml	0.091 0.102	0.096	0.000	
2.1 pmol/L	0.235 0.235	0.235	0.139	
6.0 pmol/L	0.501 0.499	0.500	0.404	
17.3 pmol/L	1.446 1.076	1.261	1.165	
54.0 pmol/L	3.123 3.107	3.115	3.019	
Control I	0.374 0.373	0.374	0.278	4.1
Control II	1.019 1.020	1.020	0.924	13.7



EXPECTED VALUES

Each laboratory should establish it own normal range by using samples collected from healthy donors. Please be note that the normal range will vary depending on whether samples were collected from fasted or fed individuals. The table below indicates that the fed GLP-1 (7-36/9-36) levels are higher than fasted GLP-1(7-36/9-36) levels.

Donor	GLP-1 (7-36)/(9-36), pmol/L	
	Fasted	Fed
1	4.94	6.04
2	12.04	15.09
3	10.31	13.43
4	0.85	2.1
5	0.25	3.93
6	2.09	6.46
7	0.52	1.55
8	18.53	19.04
9	15.87	24.72
10	1.12	4.92

LIMITATIONS OF THE PROCEDURE

1. Since there is no Gold Standard concentration or international standard available for GLP-1 (7-36/9-36) 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. If a sample has a value higher than the level 5 standard, it is recommended to dilute the sample with an appropriate GLP-1 free human plasma matrix or an appropriate buffer matrix (e.g., zero standard), and then assay again for a more accurate report.
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) or (9-36) levels.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity of this GLP-1 (7-36/9-36) ELISA was determined by calculating the mean +3 standard deviations for 12 duplicates of the zero standard. The sensitivity of the assay is 0.6 pmol/L.

Specificity

This assay has been tested and exhibits the following cross-reactivity:

GLP-1 (7-36)	100%
GLP-1 (9-36)	100%
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 of two control samples in a single assay.

#	Average GLP-1 (7-36/9-36) (n = 8)	SD	CV
Sample 1	3.02 pmol/L	0.11	3.7%
Sample 2	10.20 pmol/L	0.48	4.7%

The inter-assay precision was determined by 8 individual assays on different dates with two control samples.

#	Average GLP-1 (7-36/9-36) (n = 8)	SD	CV
Sample 1	4.16 pmol/L	0.26	6.2%
Sample 2	12.58 pmol/L	1.20	9.5%

Linearity

Two samples were diluted 1:2, 1:4, and 1:8 with the zero standard. These diluted samples were measured in this assay and the linear recovery was calculated to be 91.2% to 102%.

Spike Recovery

Samples with differing concentrations of GLP-1 were combined and the percent recovery was calculated to be from 114% to 120%.

REFERENCES

1. Levy JC. Therapeutic intervention in the GLP-1 pathway in Type 2 diabetes. *Diabet Med.* 2006 Mar; 23 Suppl 1:14-9.

2. Mannucci E, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, Ciani S, Fanelli A, Messeri G, Rotella CM. Glucagon-like peptide (GLP)-1 and leptin concentrations in obese patients with Type 2 diabetes mellitus. *Diabet Med.* 2000 Oct; 17(10):713-9.

3. Nauck MA, Weber I, Bach I, Richter S, Orskov C, Holst JJ, Schmiegel W. Normalization of fasting glycaemia by intravenous GLP-1 ([7-36 amide] or [7-37]) in type 2 diabetic patients. *Diabet Med*. 1998 Nov; 15(11):937-45.

4. Byrne MM, Göke B. Human studies with glucagon-like-peptide-1: potential of the gut hormone for clinical use. *Diabet Med.* 1996 Oct; 13(10):854-60.

5. Mannucci E, Tesi F, Bardini G, Ognibene A, Petracca MG, Ciani S, Pezzatini A, Brogi M, Dicembrini I, Cremasco F, Messeri G, Rotella CM. Effects of metformin on glucagon-like peptide-1 levels in obese patients with and without Type 2 diabetes. *Diabetes Nutr Metab.* 2004 Dec; 17(6):336-42.

Short Assay Protocol:

- Add 100 µl/well of standards, controls, and samples
- Add 100 µl/well of Antibody Mixture
- Incubate 20 - 24 hours at 2-8°C, static
- Wash strips 5 times with diluted Wash Buffer
- Add 200 µl/well of TMB substrate
- Incubate 20 minutes at RT, static
- Add 50 µl/well of Stop Solution
- Read strips within 10 minutes at OD 450 nm/620 nm