Epitope Diagnostics, Inc.

EDI™ Total GLP-1 ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the quantitative measurement of the sum level of Glucagon-like peptide-1 (7-36) and (9-36)

REF **KT 602** EU: IVD CE Σ 96 +2 +8 °C

I. INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of the sum value of glucagon-like peptide-1 (7-36) and (9-36) [GLP-1 (7-36)] and (9-36) [GLP-1 (9-36)] in plasma samples. It is for *in vitro* diagnostic use only.

II. ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of GLP-1 (7-36) and (9-36) in plasma sample. The assay utilizes the two-site "sandwich" technique with two selected GLP-1 antibodies. This assay uses the same assay calibrators and tracer antibodies as the Active GLP-1 (7-36) ELISA (EDI™ catalog: KT-871).

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 specific antibody and a horseradish peroxidase (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 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)/(9-36) on the wall of the microtiter well is directly proportional to the amount of Total GLP-1 in the sample.

III. 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 8 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 expiration date on the kit box.

2. **Total GLP-1 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 Total GLP-1 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 expiration date on the kit box.

3. **Total GLP-1 Capture Antibody (Cat. No. 30361)**
One vial containing 0.6 mL of biotinylated Total GLP-1 specific antibody. It should be used only after being mixed with Total 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 expiration date on the kit box.

4. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle containing 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 expiration date on the kit box.

5. **ELISA HRP Substrate (Cat. No. 10020)**
One bottle containing 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. **ELISA Stop Solution (Cat. No. 30357)**
One bottle containing 12 mL of sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

7. **GLP-1 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 vials for exact concentration for each standard.** These reagents should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

8. **GLP-1 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 vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

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 expiration date on the kit box.

IV. SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic 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.

V. 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. Plastic microtiter well cover or polyethylene film.
9. ELISA plate shaker.
10. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
11. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.
12. DPP-4 Inhibitor.

VI. SPECIMEN COLLECTION

(1) No special preparation of the individual is necessary prior to specimen collection. However, fasting sample and non-fasting/glucose induced sample may present great significance for Total GLP-1 (7-36)/(9-36) level.

(2) Samples should not be taken from patients taking biotin-containing multivitamins or dietary supplements at least 48 hours prior to specimen collection.

(3) EDTA Plasma collection tube such as Vacutainer® BD366643 or similar products must be used for sample collection.

(4) Whole blood should be collected into a 10 mL lavender top Vacutainer® EDTA-plasma tube. Process the whole blood to get plasma specimen according to the Vacutainer® manufacturer's instruction.

(5) It is optional to immediately add 100 µL of DPP-4 inhibitor (Cat. 30380) to the collected EDTA whole blood right after the collection (**within 30 seconds**). Invert tube to mix well and place the tube on ice bath.

(6) 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. Avoid freeze-thaw until perform this test for sample Total GLP-1 (7-36)/(9-36) measurement.

VII. 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) ELISA Wash Concentrate 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.

(4) 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	

(5) Prepare Total GLP-1 Antibody Mixture: mixing Total GLP-1 Tracer Antibody and Total GLP-1 Capture Antibody by 1:21 fold dilution of the Tracer Antibody (Cat. 30360) and by 1:21 fold dilution of the biotinylated Capture Antibody (Cat. 30361) 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.

2. Assay Procedure

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

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

(3) Add **100 µL** of Total GLP-1 Antibody Mixture to each well

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

(5) 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.

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

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

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

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

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

3. 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. For samples with concentration higher than standard level 5, it is recommended to measure diluted specimen with an appropriate GLP-1 free human plasma matrix or an appropriate buffer matrix (e.g. standard zero) for a more accurate report.
3. To assure the validity of the results, each assay should include adequate controls with known Total GLP-1 (7-36)/(9-36) levels.
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 reading.

9. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

VIII. INTERPRETATION OF RESULTS

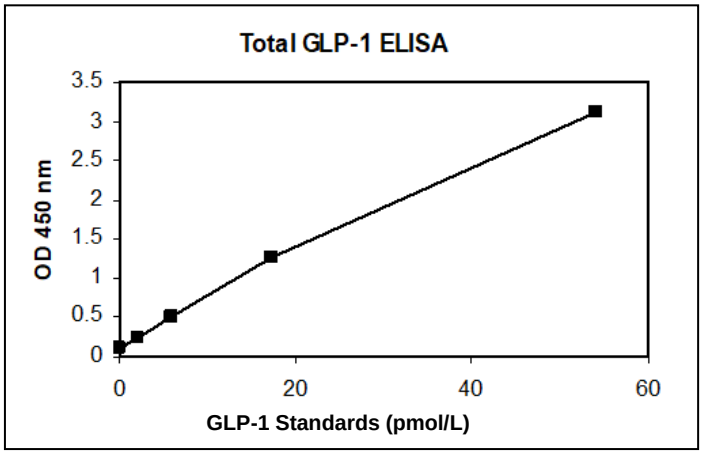
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain the corrected absorbance.
3. The standard curve is generated by the corrected absorbances of all standard levels on the ordinate against the standard concentration 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. We recommend using **Point-to-Point** or **Quadratic** curve fit.

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

IX. EXAMPLE DATA AND STANDARD CURVE

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

Well I.D.	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



X. EXPECTED VALUES

Each laboratory should establish its own normal range by using samples collected from normal, healthy individuals. Please note that the normal range is variable by using fasting samples vs. non-fasting samples. The table indicates that the fed total GLP-1 level is higher than the fasting Total GLP-1 level from normal subjects.

Donor#	Total GLP-1, pmol/L	
	Fasting	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

Based on the limited number of normal donor samples (n = 10), we found the fasting normal range is about 0.3 – 18.5 pmol/L and the fed normal range is about 2.1 – 24.7 pmol/L.

XI. LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration or international standard available for Total GLP-1 measurement, the values of assay standards were established using a highly purified GLP-1 (7-36) and (9-36) peptide and validated by Epitope Diagnostics. 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 assay standard level 5, it is recommended to measure a diluted sample for a more accurate measurement.
3. Bacterial or fungal contamination of plasma specimens 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.

XII. PERFORMANCE CHARACTERISTICS

1. Sensitivity (LoD)

The analytical sensitivity (LoD) of this Total GLP-1 ELISA as determined by 3 times the standard deviation above zero standard on 12 replicate determinations is approximately 0.6 pmol/L.

2. Specificity

This Total GLP-1 assay specifically measures GLP-1 (7-36) and (9-36). The cross reactivity of this test is listed below:

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%

3. Precision

The intra-assay precision was determined by 8 replicates for 2 control samples in a single assay. A very satisfactory within assay CV% was obtained as indicated below:

#	Average GLP-1 (7-36) (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 in different dates with 2 control samples. A satisfactory between assay CV% was observed as indicated below:

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

4. Linearity

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

5. Spike Recovery

One patient sample was spiked by another sample in the volume (200 µL + 200 µL) and measured with this assay. The spike recovery is calculated to be 114% to 120%.

XIII. WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

XIV. REFERENCES

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

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

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Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
Authorized Representative In Europe	

Total GLP-1 (7-36)/(9-36) ELISA: Condensed Assay Protocol

1. **100 µL Calibrators, controls and patient samples**
2. **100 µL Tracer and Capture Antibody Mixture**

↓ *Incubate @ 2-8 ºC for 20-24 hrs static*
Wash 5 x

3. **200 µL TMB Substrate**

↓ *Incubate @ RT for 12 min static*

4. **50 µL Stop Solution**

↓

5. **Read absorbance at 450/650 nm**