EPITOPE DIAGNOSTICS, INC.

EDI™ Active GLP-1 (7-36) Specific ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of active Glucagon-like peptide-1 (7-36) Level in Test Samples

REF KT-871 RUO 96

INTENDED USE

This high sensitive ELISA (enzyme-linked immunosorbent assay) kit is produced for the exclusively 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 use only.

ASSAY PRINCIPLE

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

Assay calibrators, 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.

1. Streptavidin Coated Microplate (10040B)

Microplate coated with streptavidin.
Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2. GLP-1 Tracer Antibody (30229)

HRP-labeled Anti-GLP-1 specific antibody in a stabilized protein matrix.
Qty: 1 x 0.6 mL
Storage: 2 – 8°C
Preparation: Must be mixed with GLP-1 (7-36) Capture Antibody (30230) and Tracer Antibody Diluent (30017) prior to use.

3. GLP-1 (7-36) Capture Antibody (30230)

Biotinylated GLP-1 (7-36) specific antibody.
Qty: 1 x 0.6 mL
Storage: 2 – 8°C
Preparation: Must be mixed with GLP-1 Tracer Antibody (30229) and Tracer Antibody Diluent (30017) prior to use.

4. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.
Qty: 1 x 30 mL
Storage: 2 –25°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.
Qty: 1 x 24 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

6. ELISA Stop Solution (30357)

1.0 M Sulfuric acid
Qty: 1 x 12 mL
Storage: 2 – 25°C
Preparation: Ready to Use.

7. GLP-1 Calibrators Levels 1 to 6 (30441-30446)

Lyophilized GLP-1 (7-36) in a liquid protein matrix with a non-azide, non-mercury based preservative. Refer to each vial for exact concentration.
Qty: 6 x Vials
Storage: 2 – 8°C (Lyophilized), <-20°C(Reconstituted) Do not exceed 3 freeze-thaw cycles.
Preparation: Must be reconstituted with 1 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

8. GLP-1 Controls (30447, 30448)

Lyophilized GLP-1 (7-36) in a liquid protein matrix with a non-azide, non-mercury based preservative. Refer to each vial for exact concentration.
Qty: 2 x Vials
Storage: 2 – 8°C (Lyophilized), <-20°C(Reconstituted) Do not exceed 3 freeze-thaw cycles.
Preparation: Must be reconstituted with 1 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

9. Tracer Antibody Diluent (30017)

For tracer antibody dilution.
Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

SAFETY PRECAUTIONS

The reagents must be used for research use only. Source material which contains reagents of bovine serum albumin 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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. 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.

SPECIMEN COLLECTION & STORAGE

1. No special preparation of individual is necessary prior to specimen collection. However, fasting sample and non-fasting/glucose induced sample may present great significance for bioactive GLP-1 (7-36) 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. **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 appropriate amount of DPP-4 inhibitor to the collected EDTA whole blood right 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 g for 10 minutes in a refrigerated centrifuge. A solid phase sample extraction procedure should be used for this type of sample before 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 (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
3. Reconstitute all calibrators (30441-30446) and controls (30447-30448) by adding **1.0 mL** of deminerialized water to each vial. Allow the calibrators and controls to sit undisturbed for 10 minutes, and then mix well by gentle vortexing. These reconstituted calibrators 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 assay.
2. It is optional to perform a solid-phase sample extraction procedure for all test specimens that are collected with DPP-IV inhibitor cocktail other than BD™ P-700 tubes. Epitope Diagnostics provides a validated and user friendly column extraction procedure and reagents packed as a GLP-1 sample extraction kit (Catalog No. KT-910).

3. Assay Procedure

1. Place a sufficient number of microwell strips (10040B) in a holder to run calibrators (30441-30446), controls (30447,30448), and samples in duplicate
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 1
C	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
D	Calibrator Level 2	Calibrator Level 6	SAMPLE 2
E	Calibrator Level 3	Control 1	SAMPLE 3
F	Calibrator Level 3	Control 1	SAMPLE 3
G	Calibrator Level 4	Control 2	SAMPLE 4
H	Calibrator Level 4	Control 2	SAMPLE 4

3. Prepare the antibody working solution by mixing GLP-1 Tracer Antibody and Capture Antibody by 1:21 fold dilution of the Tracer Antibody (30229) and by 1:21 fold dilution of the biotinylated Capture Antibody (30230) 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 and **50 µL** of the Tracer antibody in a clean test tube.
4. Add **100 µL** of run calibrators (30441-30446), controls (30447, 30448), and samples into the designated microwells.
5. Add **100 µL** of the antibody working solution. Mix by gently tapping the plate.
6. Cover the plate with one plate sealer and aluminum foil. Incubate at **2-8ºC** for **24 hours.**
7. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and then completely aspirate the contents. Alternatively, an automated microplate washer can be used.
8. Add **200 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
9. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
10. Remove the aluminum foil and plate sealer. Add **50 µL** of ELISA Stop Solution (30357) into each of the wells. Mix by gently tapping the plate.
11. Read the absorbance at **450 nm/620 nm** or **450/650 nm** within **10 minutes** with 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 calibrators, 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 calibrator, it is recommended to dilute the specimen with an appropriate GLP-1 free buffer matrix (e.g. calibrator zero) for a more accurate report.
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.

INTERPRETION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 pmol/L) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator 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.
4. The GLP-1 (7-36) concentrations for the controls and test samples are read directly from the calibration curve using their respective corrected absorbance.

LIMITATIONS 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 calibrators were established using a highly purified GLP-1 (7-36) peptide and validated by Epitope Diagnostics. Results obtained with different assay methods or kits cannot be used interchangeably.
2. For unknown sample value read directly from the assay that is greater than assay calibrator level-6, it is recommended to measure a diluted sample for 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) levels.

EXPECTED VALUES

Each laboratory should establish its own normal range by using samples collected from normal healthy people. Please note that the normal range may be variable by using fasting samples vs. non-fasting samples.

GLP-1(7-36) pg/ml = GLP-1 (7-36) pmol/l x 3.298

Based on limited number of normal donor samples (n = 10), we found the fasting normal range is about 0.5 – 3.1pmol/L and the fed normal range is about 0.6 – 16.7 pmol/L. Following table shows that the Active GLP-1 (7-36) is much higher in fed than fasting in normal donors.

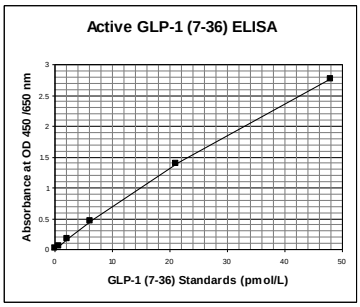
Donor Number	Active GLP-1 (7-36), pmol/L	
	Fasting	Fed
1	0.847	4.423
2	1.393	5.961
3	1.048	8.582
4	2.348	0.581
5	0.777	1.532
6	1.525	16.692
7	0.540	5.528
8	2.658	4.280
9	3.061	8.917
10	1.518	9.961

EXAMPLE DATA

A typical absorbance data and the resulting calibration 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.

Note: This example curve should not be used in lieu of calibration curve run with each assay.

Well ID	Reading Absorbance (450/650 nm)			Concentration (pmol/L)
	Readings	Average	Corrected	
Calibrator Level 1: 0 pmol/L	0.010 0.011	0.011	0.000	
Calibrator Level 2: 0.64 pmol/L	0.054 0.057	0.055	0.044	
Calibrator Level 3: 2.20 pmol/L	0.157 0.174	0.165	0.154	
Calibrator Level 4: 6.20 pmol/L	0.451 0.451	0.451	0.440	
Calibrator Level 5: 21.00 pmol/L	1.399 1.370	1.385	1.374	
Calibrator Level 6: 48.00 pmol/L	2.741 2.785	2.763	2.752	
Control 1	0.456 0.458	0.457	0.446	6.29
Control 2	0.918 0.932	0.925	0.914	13.71



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 calibrator 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%

Reproducibility and Precision
The intra-assay precision was determined by 8 replicates for two control samples in a single assay. The inter-assay precision was validated by measuring three samples in twelve (12) different assays in duplicate. The results indicate below:

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (pmol/L)	3.49	10.18	4.13	12.14
Standard Deviation	0.19	0.26	0.16	0.68
CV (%)	5.4	2.5	3.9	3.9

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

Sample Dilution format	GLP-1 (7-36) zero human serum	Observed value (pmol/L)	Expected value (pmol/L)	Linear recovery (%)
Sample 1				
50 µL	250 µL	0.64	0.70	91.3
100 µL	200 µL	1.06	1.39	76.1
150 µL	150 µL	2.21	2.12	104.3
200 µL	100 µL	2.88	2.82	102.2
250 µL	50 µL	4.18	3.55	117.9
Sample 2				
50 µL	250 µL	2.70	2.79	96.7
100 µL	200 µL	5.09	5.55	91.8
150 µL	150 µL	7.49	8.34	89.8
200 µL	100 µL	11.40	11.13	102.5
250 µL	50 µL	13.37	13.89	96.3

Spike Recovery
Patient samples were spiked each other in the sample volume (200 µL + 200 µL) and measured with this assay. The spike recovery is calculated.

Samples (pmol/L)	Samples (pmol/L)	Observed value (pmol/L)	Expected value (pmol/L)	Recovery (%)
32.45	7.49	18.56	19.98	92.9
6.67	18.62	11.25	12.65	89.0
2.40	7.61	4.12	5.00	82.4

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.

This product is developed and manufactured by

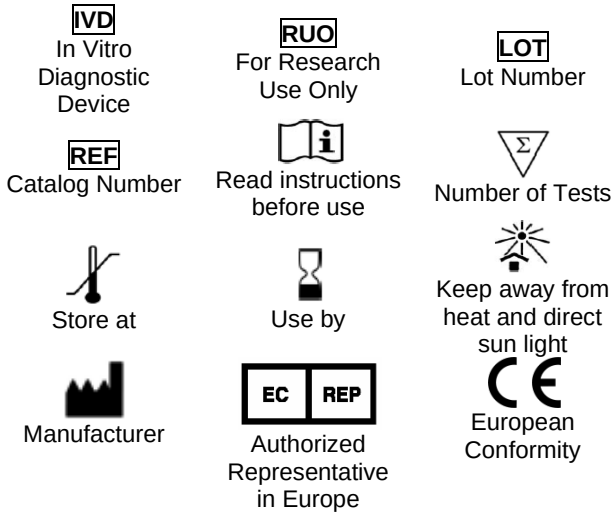
Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

EC	REP

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)



SHORT ASSAY PROCEDURE

1. Add **100 µL** of the calibrators, controls, and samples into the designated microwells.
2. Add **100 µL** of the working antibody solution.
3. Mix, cover, and incubate at **2-8 ºC** for **24 hours**.
4. Wash each well five times.
5. Add **200 µL** of substrate to each well.
6. Cover and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
7. Add **50 µL** of the stop solution to each well.
8. Read the absorbance at **450/620 nm** or **450/650 nm.**

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
info@listarfish.it
www.listarfish.it