Epitope Diagnostics, Inc.

EDI™ Rodent 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 rodent plasma samples

Catalog Number: KT 878 Store at 2 – 8 ºC Upon Receipt

For Research Use Only **Not for use in diagnostic procedures**

INTENDED USE

The primary amino acid sequence of GLP-1 peptide is identical among mammalian species, i.e. rat, mouse, pig, human, etc. This ELISA (enzyme-linked immunosorbent assay) kit is produced for the exclusively quantitative determination of bioactive glucagon-like peptide-1 (7-36) amide [GLP-1 (7-36)] level in rat and mouse plasma samples with only 20 ul of sample volume. ***This kit is for research purpose only.***

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of bioactive GLP-1 (7-36) in rodent plasma that usually have limited amount of sample available for analysis. 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 peroxidate (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. Regents from different kit lot numbers should not be combined or interchanged.

1. **Streptavidin Coated Microplate (Cat. No. 10040)**
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 expiration date on the kit box.

2. **GLP-1 Tracer Antibody (Cat. No. 30487)**
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 expiration date on the kit box.

3. **GLP-1 (7-36) Capture Antibody (Cat. No. 30488)**
One vial containing 0.6 mL of biotinylated GLP-1 (7-36) specific antibody. It should be used only after 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 expiration date on the kit box.

4. **ELISA 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 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 contains 12 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. 10030)**
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 expiration date on the kit box.

7. **GLP-1 Standards (Cat. No. 30501 – 30507)**
Seven 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 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. 30508 – 30509)**
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. 30489)**
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.

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) was derived in the contiguous 48 United States. It was obtained only from donor healthy 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 potential 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 sever 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 20 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 multi-channel 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

1. No special preparation of animal 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. **BD™ P700** Blood Collection and Preservation System (contains a DPP-4 protease inhibitor cocktail) must be used for sample collection.
3. 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 immediately after the collection (**within 30 seconds**). Refer to DPP-4 inhibitor manufacturer's instruction. Invert tube several times to mix well and place the tube in an ice bath. Centrifuge the tube at 1000 g for 10 minutes in a refrigerated centrifuge..
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 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. Regents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior 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

For **direct** measuring Active GLP-1 (7-36), **BD™ P-700 Blood Collection and Preservation System** must be used for sample collection. There is no other sample preparation necessary prior to assay.

3. Assay Procedure

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

(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	C II
B	STD 1	STD 5	C II
C	STD 2	STD 6	SAMPLE 1
D	STD 2	STD 6	SAMPLE 1
E	STD 3	STD 7	SAMPLE 2
F	STD 3	STD 7	SAMPLE 2
G	STD 4	C I	SAMPLE 3
H	STD 4	C I	SAMPLE 3

(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 (30487) and by 1:21 fold dilution of the biotinylated Capture Antibody (30488) with the Tracer antibody Diluent. For each strip, it is required to mix 1 mL of the Tracer Antibody Diluent (30489) with 50 µL the Capture Antibody and 50 µL of the Tracer Antibody in a clean test tube.

(4) Add **20 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 **100 µL** of ELISA HRP Substrate 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 **100 µL** of ELISA Stop Solution 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.

Well I.D.	OD 450 nm/650 nm Absorbance			Results (pM)
	Readings	Average	Corrected	
0 pmol/mL	0.029 0.030	0.030	0.000	
0.85 pM	0.065 0.064	0.065	0.036	
2.4 pM	0.111 0.104	0.108	0.078	
7.9 pM	0.200 0.192	0.196	0.166	
24 pM	0.414 0.516	0.465	0.435	
54 pM	1.051 1.075	1.063	1.033	
146 pM	2.539 2.583	2.561	2.531	
Control I	0.161 0.161	0.161	0.131	5.74
Control II	0.310 0.365	0.337	0.307	16.35

2. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate sample should be used for data reduction and the calculation of results.
3. For samples with higher than level 7 standard, it is recommended to measure diluted the specimen with an appropriate GLP-1 free buffer matrix (e.g. standard zero) for a more accurate measurement.
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 use. Avoid foaming.
10. For linearity test, optimal dilution buffer matrix should be used to achieve satisfactory linear recovery.
11. Different dilution buffer matrix used for calibrator or rodent sample may show different linear recovery.

INTERPRETATION OF RESULTS

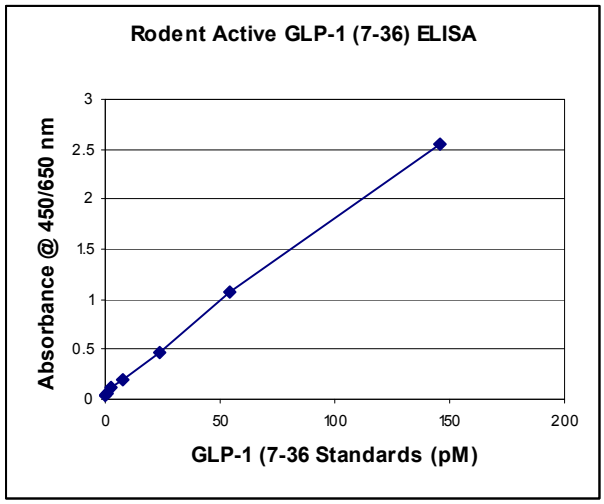
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbances of all standard levels 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 **log-log** 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. If log-log graphic paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the 2nd standard and the next highest standard should be calculated by the formula:

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

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.**



EXPECTED VALUES

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

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

Based on limited number of normal donor rodent samples (n = 9), we found the fasting normal range is about 0.5 – 3.1pM and the fed normal range is about 0.6 – 16.7 pM. The table below shows that in general the Active GLP-1 (7-36) is higher in fed than fasting samples in normal donors.

Donor#	Active GLP-1 (7-36) pM	Donor#	Active GLP-1 (7-36) pM
	Fasting		Fed
1	0.65	10	2.67
2	1.84	11	3.24
3	2.10	12	2.16
4	0.19	13	0.76
5	1.21	14	0.69
6	0.27	15	0.11
7	0.39	16	1.02

8	0.64	17	1.09
9	0.05	18	0.49

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 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 standard level-7, it is recommend measuring 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.

PERFORMANCE CHARACTERISTICS

Sensitivity
The sensitivity of the rodent Active GLP-1 (7-36) ELISA is determined by 3 times the standard deviation above zero standard on 12 replicate determinations is approximately 0.1 pM.

Specificity
This Bioactive GLP-1 (7-36) assay is specific measure 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.

#	Average GLP-1 (7-36) pM (n = 12)	SD	CV
Sample 1	4.09	0.274	6.7%
Sample 2	20.56	1.625	7.9%

The inter-assay precision was determined by 6 separate assays on different days with two control samples. The result for between assay CV% was observed as indicated below.

#	Average GLP-1 (7-36) pM (n = 4)	SD	CV
Sample 1	5.53	0.50	9.1%
Sample 2	16.73	0.94	5.6%

Spike Recovery

Rat plasma samples were spiked with 5-20 pM GLP-1 peptide, and the spike recovery was calculated. Please note that matrix used to prepare the spiked samples may exhibit matrix effects.

Sample (pM)	Spike (pM)	Measured value (pM)	Expect value (pM)	Recovery (%)
2.676	5.0	7.42	7.68	96.7
	10.0	11.27	12.68	88.9
	20.0	19.97	22.68	88.0
3.944	5.0	7.38	8.94	82.5
	10.0	13.94	13.89	99.6
	20.0	24.59	23.94	102.7

Linearity
Two rat plasma samples were diluted with GLP-1 (7-36) standard matrix at various percentages. These diluted samples were measured in this assay and the linear recovery was calculated.

Sample 1

Sample / matrix	GLP-1 pM	% Recovery
100% / 0%	107.89	100.0%
90% / 10%	88.20	90.8%
80% / 20%	82.26	95.3%
70% / 30%	65.26	86.4%
60%/ 40%	55.99	86.5%
50% / 50%	44.91	83.3%
40% / 60%	39.16	90.7%
30% / 70%	30.54	94.3%
20% / 80%	22.00	101.9%
10% / 90%	10.33	95.8%

Sample 2

Sample / matrix	GLP-1 pM	% Recovery
100% / 0%	65.50	100%
90% / 10%	54.67	92.7%
80% / 20%	53.69	102.5%
70% / 30%	49.24	107.4%
60%/ 40%	41.52	105.7%
50% / 50%	34.78	106.2%
40% / 60%	30.21	115.3%
30% / 70%	24.45	124.5%
20% / 80%	15.39	117.5%
10% / 90%	6.90	105.3%

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.

REFERENCES

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Abbreviated Assay Protocol:

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

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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