GLP-1 ELISA RUO

AL-172

INTENDED USE

The Glucagon-like peptide-1 (GLP-1) enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of GLP-1 in plasma and other biological fluids. This kit is intended for Research Use Only and is not for use in diagnostic or therapeutic procedures.

SUMMARY AND EXPLANATION

Glucagon-like peptide-1 (GLP-1) is a 30-amino acid gut hormone secreted from the intestinal L-cells. The GLP-1 sequence is highly conserved among mammals and is released in its active form (7-36) in response to food intake. It is quickly degraded into its inactive form (9-36) after selective cleavage by dipeptidyl peptidase-4 (DPP-4) and is the major circulating form. GLP-1 reduces glucose levels by regulating pancreatic secretion, slowing gastric emptying and lowering the desire for food intake. Therefore, it is an important biomarker for the study of diabetes and obesity[1-5].

PRINCIPLE OF THE TEST

The GLP-1 ELISA is a quantitative three-step sandwich type immunoassay. In the first step the samples are incubated on a solid phase extraction plate to remove proglucagon fragments that are attached as an extension to GLP-1. In the second step the Calibrators, Controls and Unknown (samples from solid phase extraction plate) are added to GLP-1 antibody coated microtiter wells and incubated with biotinylated GLP-1 antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The biotin-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of GLP-1 in the samples, calibrators and controls. This kit is designed to measures total of GLP-1 with absolute specificity.

MATERIALS SUPPLIED

CAL-172A - CAL-172F GLP-1 Calibrators A – F (Lyophilized)

Five vials, labeled A-F, containing concentrations of approximately 0-212 pg/mL GLP-1 in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use and discard after the run. Avoid repeated freeze thaws. The GLP-1 concentration in the calibrators is traceable to the manufacturer's working calibrators (peptide weight/volume) and is **corrected for the dilution factor generated during solid phase extraction procedure**. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

CTR-172-I & CTR-172-II GLP-1 Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high GLP-1 concentrations in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use and discard after the run. Avoid repeated freeze thaws.

PLT-172 GLP-1 Antibody Coated Microtitration strips

One strip -holder, containing 12 strips and 96 microtitration wells with anti GLP-1 antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

PLT-SPE-01 Solid Phase Extraction Plate

One strip holder, containing 12 strips and 96 microtitration wells with antibody nonspecific to GLP-1 immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

ASB-174 Proglucagon Assay Buffer

One bottle, 8 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

BCC-172 GLP-1 Biotin Conjugate Concentrate

One vial, 0.4 mL containing biotinylated anti-GLP-1 antibody in a protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

CND-172 GLP Conjugate Diluent

One bottle, 12 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SAR-172 GLP-1 Streptavidin-Enzyme Conjugate-Ready-To-Use (RTU)

One bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store at 2-8°C until expiration date.

TMB-100 TMB Solution

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2-30°C until expiration date.

WSH-100 Wash Concentrate A

One bottle, 60 mL, containing buffered saline with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate absorbance reader capable of measuring absorbance at 450 nm, 405 nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–1000 µL.
5. Multi-channel pipette to deliver 20-250 µl.
6. Vortex mixer.
7. Deionized water.
8. Repeater Pipette

WARNINGS AND PRECAUTIONS

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

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations

WARNING: Potential Biohazardous Material

This reagent may contain some animal source material (e.g. BSA) or materials used in conjunction with animal source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5th Edition, 2007.[6]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[7] as a preservative. Pro-Clean 400 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) K_2EDTA Plasma is the recommended sample type.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.

d) Avoid assaying lipemic, hemolyzed or icteric samples.

e) Avoid repeated freezing and thawing of samples.

f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the GLP-1 ELISA assay. It is the user's responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix and match different reagent lots and use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. Care should be taken to add TMB into the wells to minimize potential assay drift due to variation in the TMB incubation time. Avoid exposure of the reagents to excessive heat or direct sunlight.

PREPARATION OF REAGENTS

1. **GLP-1 Calibrators B-F and GLP-1 Controls I & II**: Tap and reconstitute GLP-1 Calibrators B-F and GLP-1 Controls I & II each with **1 mL** deionized water and solubilize for 10 minutes, vortex and use.
2. **GLP-1 Antibody Biotin Conjugate Solution:** The GLP-1 antibody Biotin Conjugate Concentrate should be diluted at a ratio of **1 part into 50 parts** of the GLP conjugate diluent, according to the number of wells used. For an entire plate, pipet exactly 120 µL of the Biotin Conjugate Concentrate into 6 mL of the Proglucagon conjugate diluent.
3. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature when stored in a tightly sealed bottle.
4. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, Controls, and unknowns should be assayed in duplicate.

Note: DO NOT TREAT GLP-1 CALIBRATORS AND CONTROLS ON PLT-SPE-01.

1. Label the PLT-SPE-01 microtitration strips to be used in the order in which the samples will be transferred to the GLP-1 (PLT-172) plate.
2. Pipette **50 µL** of samples on PLT-SPE in the designated wells.
3. Add **50 µL** of proglucagon **assay buffer** (ASB-174) to the PLT-SPE-01 plate.
4. Incubate the PLT-SPE-01 plate, shaking at a fast speed (600-800 rpm) on an orbital microplate shaker, for 60 minutes at room temperature (23 ± 2°C). **DO NOT WASH THE PLATE.**
5. Label the PLT-172 microtitration strips to be used in the order in which the samples will be transferred from PLT-SPE-01 plate.
6. Pipette **50 µL** of the **Calibrators, Controls** (See preparation of reagent section) **on GLP-1 plate (PLT-172)** to the appropriate wells of GLP-1 plate. **Transfer 50 µL the samples from PLT-SPE-01after incubation (From step#4) to designated wells on PLT-172 using precision pipette.**
7. Add **50 µL** of the **GLP-1 Antibody Biotin Conjugate Solution** (see preparation of reagent section) to each well using a repeater pipette.
8. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
9. Aspirate and wash each strip **5 times** (**350 µL/per well)** with **Wash Solution** using an automatic microplate washer.
10. Add **100 µL** of the **Streptavidin-Enzyme Conjugate-RTU** to each well using a repeater pipette.
11. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
12. Aspirate and wash each strip **5 times** (**350 µL/per well)** with the **Wash Solution** using an automatic microplate washer.

13. Add **100 µL** of the **TMB chromogen** solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
14. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).
    NOTE: Visually monitor the color development to optimize the incubation time.
15. Add **100 µL** of the **Stopping Solution** to each well using a repeater pipette. Read the absorbance of the solution in the wells within **20 minutes**, using a microplate reader set to **450 nm**.

NOTE: *Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.*

Note: *For rat and mice samples dilute the specimen in Calibrator A. (1 part of plasma in 4 parts of diluent) and follow the procedure.*

RESULTS

NOTE: *The results in this package insert were calculated by plotting the* ***log optical density (OD) data on the y-axis and log GLP-1 concentration on X-axis*** *using a cubic regression curve-fit. Alternatively, linear regression curve-fit can be used. Other data reduction methods may give slightly different results.*

1. Calculate the mean optical density (OD) for each calibrator, Control, or Unknown.
2. Optimum results can be obtained at incubation temperature of (23 ± 2°C).
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the GLP-1 concentrations in pg/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the GLP-1 concentrations of the Controls and unknowns from the calibration curve by matching their mean OD readings with the corresponding GLP-1 concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 pg/mL (Cal. A) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a **dilution factor**, if applicable.

LIMITATIONS

The reagents supplied in this kit are optimized to measure total GLP-1 levels in human EDTA plasma. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.[8]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- GLP-1 ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for GLP-1 controls are printed on the Calibration Card.
- A full calibration curve, low and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean Absorbance	Conc. (pg/mL)
	Calibrators	0.015 (Blank)	0
A1, A2	A		
B1, B2	B	0.035	15.0
C1, C2	C	0.145	36.6
D1, D2	D	0.581	80.6
E1, E2	E	1.089	118.4
F1, F2	F	2.995	213.0

CAUTION: *The above data must not be employed in lieu of data obtained by the user in the laboratory.*

ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in pg/mL.
1 pg/mL = 0.32 pmol/L

Analytical Sensitivity:

The analytical sensitivity in the GLP-1 ELISA assay, as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 pg/mL) and calibrator B (15 pg/mL), Analytical sensitivity is **1.9 pg/mL**.

Linearity:

EDTA plasma samples containing various GLP-1 levels were diluted in GLP-1 Calibrator A (CAL-172A) and then added to PLT-SPE-01 and run as per the assay procedure. The % recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
GLP-1 Ag	Neat	106.50	NA	NA
	1:2	53.25	54.30	102%
	1:4	26.62	25.78	97%
	1:8	13.31	12.83	96%
	1:32	6.66	6.74	101%
Sample-1	Neat	56.83	NA	NA
	1:2	28.42	26.65	94%
	1:4	14.21	13.30	94%
	1:8	7.10	6.55	92%
Sample-2	Neat	26.73	NA	NA
	1:2	13.36	13.30	90%
	1:4	6.68	7.22	108%

Imprecision:

Reproducibility of the GLP-1 assay was determined in a study using two kit controls and three plasma QC samples. The study included a total of 9 assays, replicates of three per assay (n=27). Representative data were calculated and are presented in the following table.

Sample	Mean Conc.	Within Run		Between Run		Total	
	(pg/mL)	SD	%CV	SD	%CV	SD	%CV
Control I	22.06	0.84	3.8%	1.36	6.2%	1.60	7.2%
Control II	52.74	2.47	4.7%	0.00	0.0%	2.47	4.7%
QC1	13.67	0.44	3.2%	0.51	3.8%	0.68	5.0%
QC2	25.50	0.73	2.8%	1.09	4.3%	1.31	5.1%
QC3	121.67	2.86	2.3%	9.15	7.5%	9.58	7.9%

Recovery:

Known amounts of GLP-1 was added to two samples containing different levels of GLP-1. The concentration of GLP-1 was determined before and after the addition of exogenous GLP-1 and the percent recovery was calculated.

Sample	Endogenous Conc. (pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	17.93	26.78	24.54	92%
		35.64	28.67	80%
		44.50	33.14	74%
2	17.35	26.27	25.70	98%
		35.18	28.01	80%

Analytical Specificity:

This assay is calibrated against human -GLP-1 (9-36) and cross-reactivity to other closely related analytes as listed below.

Cross-Reactant	Concentration	% Cross-reactivity
Insulin	10 ng/mL	ND
Thyroglobulin	10 ng/mL	ND
C-Peptide	10 ng/mL	ND
GLP-2	1000 ng/mL	ND
GRPP	1000 ng/mL	ND
Oxyntomodulin	1000 ng/mL	ND
Glucagon	1000 ng/mL	ND
Glicentin	1000 ng/mL	ND
MPGF-1	1 ng/mL	ND
GLP-1 (7-36)	100 pg/mL	22.6%
GLP-1 (9-36)	100 pg/mL	100%
GLP-1 (1-36)	5 ng/mL	4.4%

ND=Non-Detectable.

REFERENCES

1. Gareth E. Lim and Patricia L. Brubaker., Glucagon-Like Peptide 1 Secretion by the L-Cell. Diabetes 2006 Dec; 55(Supplement 2): S70-S77.
2. Holst, Jens J. et al., Glucagon-like peptide-1, glucose homeostasis and diabetes. Trends in Molecular Medicine 2008; 14(4): 161 – 168.
3. Bak MJ, et.al., Specificity and sensitivity of commercially available assays for glucagon-like peptide-1 (GLP-1): implications for GLP-1 measurements in clinical studies. Diabetes Obes Metab. 2014 Nov;16(11):1155-64.
4. Caporarello N,et.al., Insulin receptor signaling and glucagon-like peptide 1 effects on pancreatic beta cells. PLoS One. 2017 Aug 2;12(8): 1-11.
5. Lee YS, Jun HS. Anti-diabetic actions of glucagon-like peptide-1 on pancreatic beta-cells. Metabolism. 2014 Jan;63(1):9-19.
6. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5
7. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available: http:// www.cdc.gov/niosh.
8. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures.

Manufactured by:
Ansh Labs
445 Medical Center Blvd.
Webster, TX 77598-4217, U.S.A.