Datasheet for ABIN1305171

GLP-1 ELISA Kit

1 Image | 1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	GLP-1
Binding Specificity:	AA 7-36, active
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.05-48 pM/L
Minimum Detection Limit:	0.05 pM/L
Application:	ELISA

Product Details

Purpose:	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 purposes only.
Brand:	ED™
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This Bioactive GLP-1 (7-36) assay is a specific measure of GLP-1 (7-36).
Cross-Reactivity (Details):	It is expected that this assay does not detect following peptides. GLP-1 (7-36) 100 % GLP-1 (9-

Product Details

	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 %
Components:	1. Streptavidin Coated Microplate 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
Material not included:	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.

Target Details

Target:	GLP-1
Alternative Name:	GLP-1 (GLP-1 Products)

Application Details

Comment:	Active GLP-1 ELISA
Assay Time:	4 h
Plate:	Pre-coated
Protocol:	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 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 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.
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 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.
Sample Collection:	(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) 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. (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 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. (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.
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).

Application Details

Assay Procedure:	(1) Place a sufficient number of streptavidin-coated microwell strips/wells (Cat. 10040B) in a holder to run GLP-1 (7-36) standards, controls and unknown samples in duplicate. (2) Test Configuration (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 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 with 50 µL the Capture Antibody and 50 µL of the Tracer Antibody in a clean test tube. (4) Add 100 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 200 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 50 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.
Calculation 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 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.
Assay 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. The inter-assay precision was determined by 13 individual assays in different dates with two control samples. A very satisfactory between assay CV% was observed as indicated below.
Restrictions:	For Research Use only

Handling

Precaution of Use:	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.
Storage:	4 °C

Publications

Product cited in:

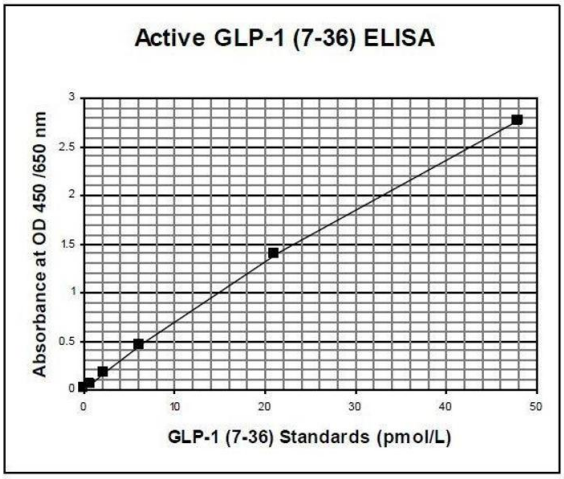
Covey, Putta, Cesano: "Single cell network profiling (SCNP): mapping drug and target interactions." in: **Assay and drug development technologies**, Vol. 8, Issue 3, pp. 321-43, (2010) (PubMed).

Critchley-Thorne, Simons, Yan, Miyahira, Dirbas, Johnson, Swetter, Carlson, Fisher, Koong, Holmes, Lee: "Impaired interferon signaling is a common immune defect in human cancer." in: **Proceedings of the National Academy of Sciences of the United States of America**, Vol. 106, Issue 22, pp. 9010-5, (2009) (PubMed).

Krutzik, Crane, Clutter, Nolan: "High-content single-cell drug screening with phosphospecific flow cytometry." in: **Nature chemical biology**, Vol. 4, Issue 2, pp. 132-42, (2008) (PubMed).

Perez, Mitchell, Campos, Gao, Li, Nolan: "Multiparameter analysis of intracellular phosphoepitopes in immunophenotyped cell populations by flow cytometry." in: **Current protocols in cytometry / editorial board, J. Paul Robinson, managing editor ... [et al.]**, Vol. Chapter 6, pp. Unit 6.20, (2008) (PubMed).

Irish, Kotecha, Nolan: "Mapping normal and cancer cell signalling networks: towards single-cell proteomics." in: **Nature reviews. Cancer**, Vol. 6, Issue 2, pp. 146-55, (2006) (PubMed).



ELISA

Image 1.