Datasheet for ABIN2014343

GLP-1 ELISA Kit

1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	GLP-1
Binding Specificity:	AA 7-36, active
Reactivity:	Rodents
Method Type:	Sandwich ELISA
Detection Range:	0.1-146 pM
Minimum Detection Limit:	0.1 pM
Application:	ELISA

Product Details

Purpose:	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.
Brand:	EDI™
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This Bioactive GLP-1 (7-36) assay is specific measure GLP-1 (7-36).

Product Details

Cross-Reactivity (Details):	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 %
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 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

Target Details

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

Application Details

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 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.
Reagent Preparation:	rior 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.
Sample 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.
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.
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 (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
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 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: Corrected absorbance (unknown) Value of unknown = x Value of the 2nd STD Corrected Absorbance (2nd STD)
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 6 separate assays on different days with two control samples.

Application Details

	The result for 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) 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.
Storage:	4 °C

Publications

Product cited in:	Wang, Park, La Marca, Than, Lin: "BMP-2 inhibits tumor-initiating ability in human renal cancer stem cells and induces bone formation." in: **Journal of cancer research and clinical oncology**, Vol. 141, Issue 6, pp. 1013-24, (2015) (PubMed). Chanchareonsook, Tideman, Feinberg, Jongpaiboonkit, Lee, Flanagan, Krishnaswamy, Jansen: " Segmental mandibular bone reconstruction with a carbonate-substituted hydroxyapatite-coated modular endoprosthetic poly(?-caprolactone) scaffold in Macaca fascicularis." in: **Journal of biomedical materials research. Part B, Applied biomaterials**, Vol. 102, Issue 5, pp. 962-76, (2014) (PubMed). Clark, Milbrandt, Hilt, Puleo: "Mechanical properties and dual drug delivery application of poly(lactic-co-glycolic acid) scaffolds fabricated with a poly(?-amino ester) porogen." in: **Acta biomaterialia**, Vol. 10, Issue 5, pp. 2125-32, (2014) (PubMed). Alegre-Aguarón, Sampat, Xiong, Colligan, Bulinski, Cook, Ateshian, Brown, Hung: "Growth factor priming differentially modulates components of the extracellular matrix proteome in chondrocytes and synovium-derived stem cells." in: **PLoS ONE**, Vol. 9, Issue 2, pp. e88053, (

2014) (PubMed).

Cushnie, Ulery, Nelson, Deng, Sethuraman, Doty, Lo, Khan, Laurencin: "Simple signaling molecules for inductive bone regenerative engineering." in: **PLoS ONE**, Vol. 9, Issue 7, pp. e101627, (2014) (PubMed).