Datasheet for ABIN5564553

GLP-2 ELISA Kit

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Overview

Quantity:	96 tests
Target:	GLP-2
Reactivity:	Human, Pig, Rat, Monkey
Method Type:	Sandwich ELISA
Detection Range:	0.281-72 ng/mL
Minimum Detection Limit:	0.281 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Glucagon-like Peptide 2 (GLP-2) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of GLP-2 in plasma, serum, and cell culture samples. The kit has been validated using rat, human, swine, and monkey samples. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures GLP-2 in approximately 4 hours. A polyclonal antibody specific for GLP-2 has been pre-coated onto a 96-well microplate with removable strips. GLP-2 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for GLP-2, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	GLP-2 Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against GLP-2. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. GLP-2 Standard: GLP-2 in a buffered protein base (108 ng, lyophilized). Biotinylated GLP-2 Antibody (50x): A 50-fold biotinylated polyclonal antibody against GLP-2 (120 l). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (20 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 l). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water Incubator (37 °C)

Target Details

Target:	GLP-2
Abstract:	GLP-2 Products
Background:	Glucagon-like peptide 2 (GLP-2) is a thirty-three amino acid peptide that is secreted by intestinal endocrine cells alongside GLP-1. GLP-2 and GLP-1 are co-secreted from enteroendocrine L-cells located in distal intestine in response to enteral nutrient ingestion, particularly fats and carbohydrates. GLP-2 secretion is mediated by direct nutrient stimulation of the L-cells and indirect action from enteroendocrine and neural inputs. GLP-2 acts to enhance nutrient absorption by inhibiting gastric motility and secretion and by stimulating nutrient transport (1).
Gene ID:	24952, 60432
UniProt:	P06883, Q9Z0W0

Application Details

Assay Time:	4 h
Plate:	Pre-coated
Protocol:	• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours. • Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour. • Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.

	• Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 15 minutes. • Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. EIA Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8 °C. GLP-2 Standard: Reconstitute the 108 ng of GLP-2 Standard with 1.5 mL of EIA Diluent to generate a 72 ng/mL standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution (72 ng/mL) 4-fold with EIA Diluent to produce 18, 4.5, 1.125, and 0.281 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at -20 °C and use within 30 days. 4 Standard Point Dilution [GLP-2] (ng/mL) P1 1 part Standard (72 ng/mL) 72.0 P2 1 part P1 + 3 parts EIA Diluent 18.0 P3 1 part P2 + 3 parts EIA Diluent 4.5 P4 1 part P3 + 3 parts EIA Diluent 1.125 P5 1 part P4 + 3 parts EIA Diluent 0.281 P6 EIA Diluent 0.000 Biotinylated GLP-2 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with EIA Diluent. Any remaining solution should be frozen at -20 °C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with EIA Diluent. Any remaining solution should be frozen at -20 °C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Sample collection and processing should be performed as quickly as possible. Keep on ice when not in use. It is recommended that protease inhibitor cocktail is added to samples, for example: o-phenanthroline 0.44 mM, EDTA 25 mM, p-hydroxy- mercuribenzoic acid 1 mM and pepstatin A 0.12 mM. The user may need to optimize concentration of above reagents. Centrifuge samples at 3000 x g for 10 minutes. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant). Serum: Samples should be collected into a serum separator tube. Sample collection and processing should be performed as quickly as possible. Keep on ice when not in use. It is recommended that protease inhibitor cocktail is added to samples, for example: o-phenanthroline 0.44 mM, EDTA 25 mM, p-hydroxy-mercuribenzoic acid 1 mM and pepstatin A 0.12 mM. The user may need to optimize concentration of above reagents. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to

Application Details

	remove debris and collect supernatants. Dilute samples if necessary. The undiluted samples can be stored at -20 °C or below. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of GLP-2 Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated GLP-2 Antibody to each well and incubate for 1 hour. Wash the microplate as described above. Add 50 l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. 5 Add 50 l of Chromogen Substrate per well and incubate for 15 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	• Calculate the mean value of the duplicate or triplicate readings for each standard and sample. • To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. • Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	This product is for Research Use Only and is not intended for use in diagnostic procedures. Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP

Handling

	conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents. The Stop Solution is an acidic solution. 2 The kit should not be used beyond the expiration date.
Storage:	4 °C,-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Images

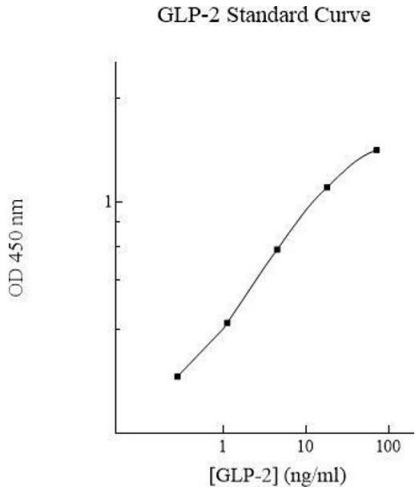

Image 1.