Datasheet for ABIN5564611

Ghrelin ELISA Kit

1 Image

Go to Product page

Overview

Quantity:	96 tests
Target:	Ghrelin (GHRL)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.25-16 ng/mL
Minimum Detection Limit:	0.25 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human Ghrelin ELISA (Enzyme-Linked Immunosorbent Assay) Kit is designed for detection of ghrelin in human plasma, serum and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human ghrelin in approximately 4 hours. A polyclonal antibody specific for human ghrelin has been pre-coated onto a 96-well microplate with removable strips. Ghrelin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human ghrelin, which is recognized by a streptavidin-peroxidase (SP) 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:	Human Ghrelin Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human ghrelin. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Ghrelin Standard: Human ghrelin in a buffered protein base (11.2 ng, lyophilized, 2 vials). Biotinylated Human Ghrelin Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human ghrelin (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). SP Conjugate (100x): A 100-fold concentrate (80 l). Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution (1x): A 0.5 N hydrochloric acid solution 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:	Ghrelin (GHRL)
Alternative Name:	Ghrelin (GHRL Products)
Background:	Ghrelin, colloquially known as the "hunger hormone," is a peptide produced in the gastrointestinal tract (1-2). It functions as a neuropeptide by regulating hunger and participating in the regulation of energy use and distribution. Higher levels of ghrelin contribute to the increase in appetite and metabolic function. Ghrelin suppression is related to weight loss (3). Like other metabolically related hormones, ghrelin is released in a circadian fashion, suggesting that ghrelin levels can indicate interruptions in circadian rhythm (4).
Gene ID:	51738
UniProt:	Q9UBU3
Pathways:	Positive Regulation of Peptide Hormone Secretion, Hormone Transport, Peptide Hormone Metabolism, Negative Regulation of Hormone Secretion, Synaptic Membrane, Feeding Behaviour

Application Details

Assay Time:	4 h
Plate:	Pre-coated

Application Details

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 25 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 to produce a 1x solution. Store for up to 30 days at 2-8 °C. Human Ghrelin Standard: Reconstitute the Human Ghrelin Standard (11.2 ng) with 0.7 mL of EIA Diluent to generate a 16 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (16 ng/mL) 2-fold with equal volume of EIA Diluent to produce 8, 4, 2, 1, 0.5, and 0.25 ng/mL solutions. EIA Diluent serves as the zero standard (0 ng/mL). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20 °C and used within 24 hours. Standard Point Dilution [Ghrelin] (ng/mL) P1 1 part Standard (16 ng/mL) 16 P2 1 part P1 + 1 part EIA Diluent 8.0 P3 1 part P2 + 1 part EIA Diluent 4.0 P4 1 part P3 + 1 part EIA Diluent 2.0 P5 1 part P4 + 1 part EIA Diluent 1.0 P6 1 part P5 + 1 part EIA Diluent 0.5 P7 1 part P6 + 1 part EIA Diluent 0.25 P8 EIA Diluent 0.0 5 Biotinylated Human Ghrelin Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with EIA Diluent to produce a 1x solution. The undiluted antibody should be stored 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 to produce a 1x solution. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with EIA Diluent to produce a 1x solution. The undiluted conjugate should be stored 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 a protease inhibitor cocktail is added to the sample. 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 the concentration of the above reagents. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. 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 a protease inhibitor cocktail is added to sample. 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 the concentration of the above reagents. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. 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 at 4 °C to remove debris and collect supernatants. 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 Human Ghrelin Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. 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 Human Ghrelin Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour. Wash the microplate as described above. Add 50 l of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 l of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue color density develops. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. 6 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

Application Details

	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 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. The kit should not be used beyond the expiration date. 2
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.

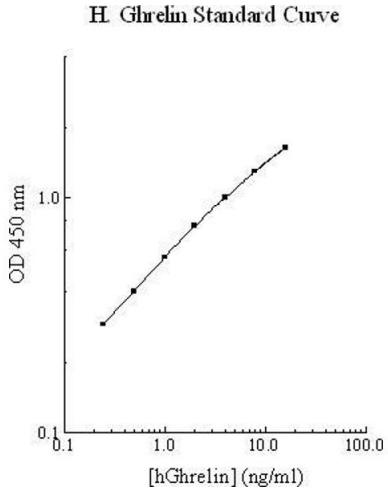
H. Ghrelin Standard Curve
OD 450 nm
1.0
0.1
0.1
1.0
10.0
100.0
[hGhrelin] (ng/ml)

Image 1.