Datasheet for ABIN5564576

Hexokinase 2 ELISA Kit

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Overview

Quantity:	96 tests
Target:	Hexokinase 2 (HK2)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	6.25-400 ng/mL
Minimum Detection Limit:	6.25 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human Hexokinase-2 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of hexokinase-2 in human plasma, serum, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human hexokinase-2 in approximately 4 hours. A polyclonal antibody specific for human hexokinase-2 has been pre- coated onto a 96-well microplate with removable strips. Hexokinase-2 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human hexokinase-2, 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. 2
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Plasma, Serum
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human Hexokinase-2 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human hexokinase-2. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human Hexokinase-2 Standard: Human hexokinase-2 in a buffered protein base (480 ng, lyophilized, 2 vials). Biotinylated Human Hexokinase-2 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human hexokinase-2 (120 l). EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (20 ml). Standard Diluent (1x): A buffered protein base with stabilizer (2 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). 3
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:	Hexokinase 2 (HK2)
Alternative Name:	Hexokinase-2 (HK2 Products)
Background:	Hexokinases (HK) catalyze the phosphorylation of hexose to produce hexose 6-phosphate. The four mammalian hexokinases are designated HK1, HK2, HK3 and HK4. Although highly conserved in amino acid sequence, these enzymes differ in molecular mass, tissue distribution, regulation, and catalytic properties. HK1 - HK3 have molecular masses of approximately 100 kDa, show broad but distinct tissue distribution, have a relatively high affinity for glucose, and are subject to feedback regulation by physiological levels of glucose 6-phosphate (G6P). The 100- kDa HK is originated from a 50- kDa precursor via gene duplication and tandem ligation. HK4, more commonly referred to as glucokinase, has a molecular mass of approximately 50 kDa. HK4 is primarily located in liver and pancreatic p-cells, has a lower affinity for glucose, and is not subject to feedback regulation by physiological levels of G6P (1). Hexokinase-2 (HK2) is the predominant hexokinase isozyme expressed in insulin-sensitive tissues, such as skeletal muscle, heart, and adipose tissue (2). It is located in the mitochondrial outer membrane. The HK2 gene encodes a 100- kDa, 917-amino acid protein that shares 94 % sequence identity with rat HK2 but only 72 % sequence identity with human HK1 (3). HK2 plays important roles in glucose catabolism and cell survival (4- 5).

Target Details

Gene ID:	3099
UniProt:	P52789
Pathways:	PI3K-Akt Signaling, Carbohydrate Homeostasis, Warburg Effect

Application Details

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 20 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 Hexokinase-2 Standard: Reconstitute the Human Hexokinase-2 Standard (480 ng) with 0.6 mL of Standard Diluent to generate an 800 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 (800 ng/mL) 2-fold with equal volume of EIA Diluent to produce 400, 200, 100, 50, 25, 12.5, and 6.25 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 48 hours. 5 Standard Point Dilution [HK2] (ng/mL) P1 1 part Standard (800 ng/mL) + 1 part EIA Diluent 400 P2 1 part P1 + 1 part EIA Diluent 200 P3 1 part P2 + 1 part EIA Diluent 100 P4 1 part P3 + 1 part EIA Diluent 50 P5 1 part P4 + 1 part EIA Diluent 25 P6 1 part P5 + 1 part EIA Diluent 12.5 P7 1 part P6 + 1 part EIA Diluent 6.25 P8 EIA Diluent 0.0 Biotinylated Human Hexokinase-2 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.

	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. 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 Hexokinase-2 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 Hexokinase-2 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that 6 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 20 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. 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.
Storage:	4 °C,-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store Standard, SP Conjugate, and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Standard Diluent (1x), 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.

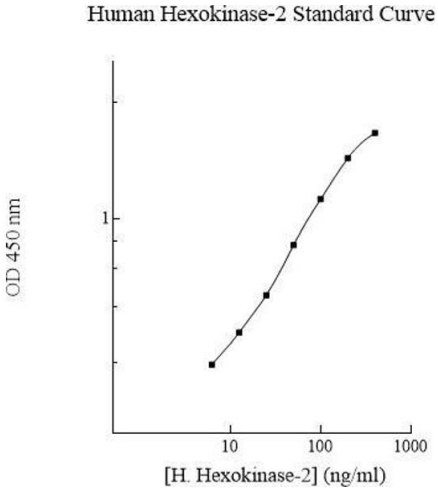

Image 1.

