ELISA PRODUCT INFORMATION & MANUAL

Human Glucokinase ELISA Kit

NBP2-60627

Enzyme-linked Immunosorbent Assay for quantitative detection of Human Glucokinase. For research use only. Not for diagnostic or therapeutic procedures.

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Assay Summary

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.

Assay Template

	A	B	C	D	E	F	G	H
1								
2								
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12								

Human Glucokinase (Hexokinase 4) ELISA Kit

Catalog No. NBP2-60627

Sample insert for reference use only

Introduction

Human glucokinase (GCK), also known as hexokinase IV or D, is a 50 kDa monomeric protein of 465 amino acids (1, 2). It is present in the liver, pancreas, small intestine, and brain. It plays important roles in glucose metabolism. In response to rising levels of glucose from eating, GCK activity increases rapidly. It catalyzes the transfer of phosphate from ATP to glucose to form glucose-6-phosphate, which is the first rate-limiting step of glycogen synthesis and glycolysis. By means of this reaction, it functions as a glucose sensor for insulin secretion in pancreatic β-cells and regulates glucose and glycogen production in the liver (3).

Principle of the Assay

The Human Glucokinase (Hexokinase 4) ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human GCK in **plasma, serum, and cell culture samples**. This assay employs a quantitative **sandwich enzyme immunoassay** technique that measures human GCK in approximately 4 hours. A polyclonal antibody specific for human GCK has been pre-coated onto a 96-well microplate with removable strips. GCK in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for GCK, 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.

Caution and Warning

- 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.

Reagents

- **Human Glucokinase Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human GCK.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Glucokinase Standard:** Human GCK in a buffered protein base (60 ng, lyophilized, 2 vials).
- **Biotinylated Human Glucokinase Antibody (60x):** A 60-fold concentrated biotinylated polyclonal antibody against GCK (100 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 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).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **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).

Storage Condition

- 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.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **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. 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 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. 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. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8°C.
- **Human Glucokinase Standard:** Reconstitute the Human Glucokinase Standard (60 ng) with 0.6 ml of **Standard Diluent** to generate a 100 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 from the standard stock solution (100 ng/ml) 2-fold with **MIX Diluent** to produce 50, 25, 12.5, 6.25, 3.125, 1.563, and 0.781 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining stock solution should be frozen at -20°C and used within 2 days. **Aliquot standard to limit repeated freeze-thaw cycles.**

Standard Point	Dilution	[GCK] (ng/ml)
P1	1 part Standard (100 ng/ml) + 1 part MIX Diluent	50
P2	1 part P1 + 1 part MIX Diluent	25
P3	1 part P2 + 1 part MIX Diluent	12.5
P4	1 part P3 + 1 part MIX Diluent	6.25
P5	1 part P4 + 1 part MIX Diluent	3.125
P6	1 part P5 + 1 part MIX Diluent	1.563
P7	1 part P6 + 1 part MIX Diluent	0.781
P8	MIX Diluent	0.0

- **Biotinylated Human Glucokinase Antibody (60x):** Spin down the antibody briefly and dilute the desired amount of the antibody 60-fold with MIX Diluent. 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.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent. The undiluted conjugate should be stored at -20°C.

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 µl of Human Glucokinase 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 µl 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 µl 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 µl of Biotinylated Human Glucokinase 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 µl of Streptavidin-Peroxidase 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 µl 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 µl 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 at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- 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.

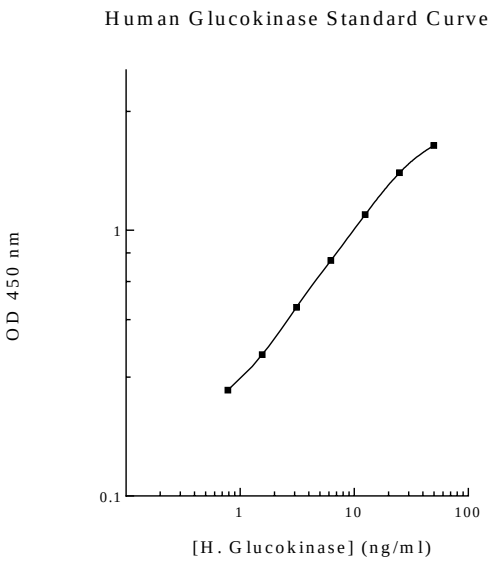
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	50	2.095 2.073	2.084
P2	25	1.651 1.636	1.644
P3	12.5	1.150 1.139	1.145
P4	6.25	0.773 0.764	0.769
P5	3.125	0.517 0.509	0.513
P6	1.563	0.343 0.337	0.340
P7	0.781	0.255 0.250	0.253
P8	0.0	0.188 0.184	0.186

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of glucokinase as calculated by 2SD from the mean of a zero standard was established to be 0.7 ng/ml.
- Intra-assay precision was determined by testing three plasma samples twenty times in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.2%	4.6%	4.7%	10.1%	9.8%	9.4%
Average CV (%)	4.2%			9.8%		

Recovery

Standard Added Value	3 – 25 ng/ml
Recovery %	90 – 111%
Average Recovery %	96%

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
1x	99%	96%
2x	102%	105%
4x	106%	107%

Cross-Reactivity

Species	**Cross Reactivity (%)**
Canine	20%
Bovine	None
Monkey	20%
Mouse	None
Rat	None
Swine	None
Rabbit	None
Protein	**Cross Reactivity (%)**
Hexokinase-1	None
Hexokinase-2	None
Hexokinase-3	<10%

Troubleshooting

Issue	**Causes**	**Course of Action**
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.

	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.