



# Human Glucokinase ELISA Kit

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Thank you for choosing Assaypro.

**Hinweis/Note:**

Der Packungsbeileger dient nur als erste Information.  
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.  
The relevant datasheet is included with the product.

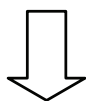
## Symbol Key



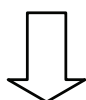
Consult instructions for use.

## Assay Summary

Add 50  $\mu$ l of Standard/ Sample per well.  
Incubate 2 hours.



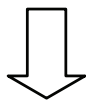
Wash, then add  
50  $\mu$ l of Biotinylated Antibody per well.  
Incubate 1 hour.



Wash, then add  
50  $\mu$ l of SP Conjugate per well.  
Incubate 30 minutes.



Wash, then add  
50  $\mu$ l of Chromogen Substrate per well.  
Incubate 30 minutes.



Add 50  $\mu$ l of Stop Solution per well.  
Read at 450 nm immediately.

# Assay Template

	1								
A	2								
B	3								
C	4								
D	5								
E	6								
F	7								
G	8								
H	9								
	10								
	11								
	12								



# AssayMax Human Glucokinase (Hexokinase 4) ELISA Kit

Catalog No. EG1001-1  
Sample Insert/Reference 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  $\beta$ -cells and regulates glucose and glycogen production in the liver (3). Mutations of the GCK gene are associated with non-insulin-dependent diabetes mellitus (4), persistent hyperinsulinemic hypoglycemia of infancy (5), and maturity-onset diabetes of younger individuals (6). GCK is a drug target for developing anti-type 2 diabetic molecules.

## Principle of the Assay

The AssayMax 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 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 then 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

- **Prepare all reagents (working 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 protocol. 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.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

## 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 (25 ng, lyophilized, 2 vials).
- **Biotinylated Human Glucokinase Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against GCK (140  $\mu$ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 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  $\mu$ 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).

## Storage Condition

- 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.

## Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20  $\mu$ l, 20-200  $\mu$ l, 200-1000  $\mu$ l, 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 assay. 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. Remove serum and assay. 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 remove debris. Collect supernatants and assay. 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 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 25 ng of Human Glucokinase Standard with 0.5 ml of MIX Diluent to generate a 50 ng/ml standard 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 solution (50 ng/ml) 1:2 with MIX Diluent to produce 25, 12.5, 6.25, 3.125, and 1.563 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 15 days.

Standard Point	Dilution	[GCK] (ng/ml)
P1	Standard (50 ng/ml)	50.00
P2	1 part P1 + 1 part MIX Diluent	25.00
P3	1 part P2 + 1 part MIX Diluent	12.50
P4	1 part P3 + 1 part MIX Diluent	6.250
P5	1 part P4 + 1 part MIX Diluent	3.125
P6	1 part P5 + 1 part MIX Diluent	1.563
P7	MIX Diluent	0.000

- **Biotinylated Human Glucokinase Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX 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 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen 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 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 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 and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.



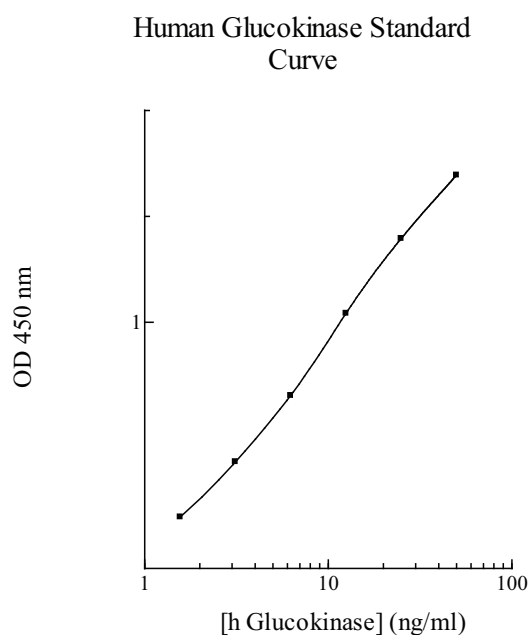
- Add 50  $\mu$ l of Chromogen Substrate per well and incubate for 30 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50  $\mu$ l of Stop Solution to each well. The color will change from blue 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.

## 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 on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

## Standard Curve

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



## Sensitivity and Specificity

- The minimum detectable dose of GCK is typically ~ 1.5 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1% and 7.4% respectively.

## Linearity

Sample Dilution	Plasma	Serum
No dilution	84%	89%
1:2	95%	98%
1:4	105%	107%

## Recovery

Standard Added Value	3 – 25 ng/ml
Recovery %	85 – 104%
Average Recovery %	96%

## Cross-Reactivity

Species	% Cross Reactivity
Canine	20%
Bovine	None
Monkey	20%
Mouse	None
Rat	None
Swine	None
Rabbit	None

## References

- (1) Stoffel M et al. (1992) Proc. Natl. Acad. Sci. U.S.A. 89:7698-7702
- (2) Tanizawa Y et al. (1992) Mol Endocrinol. 6(7):1070-1081.
- (3) Matschinsky FM (2009) Nat Rev Drug Discov. 8(5):399-416
- (4) Froguel P et al. (1992) Nature. 356(6365):162-164
- (5) Glaser B et al. (1998) N Engl J Med. 338(4):226-230
- (6) Fajans SS et al. (2001) N Engl J Med. 345(13):971-980

Version 1.7