Catalog #81509

V.6

Active GIP ELISA Kit Instructions

For the quantitative determination of active GIP
in human plasma and cell culture media

Catalog #81509
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Active GIP ELISA kit is for the quantitative determination of active GIP in human plasma and cell culture media. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid hormone part of a group of gastrointestinal hormones that cause an increase in the amount of insulin released from the beta cells of the islets of Langerhans after ingestion of food.

GIP is released from duodenal endocrine K cells after absorption of glucose or fat. GIP is a potent releaser of insulin in experimental animals and in humans provided that the blood glucose is above basal level. Recent studies demonstrate GIP may have a lot of physiological effects in addition to their glucoregulatory effects.

GIP is rapidly inactivated by DPP-4 enzyme to GIP (3-42) with a blood half-life of only several minutes.

C. Principle of the Assay

The Active GIP ELISA kit is based on a sandwich enzyme immunoassay. The 96-well plate is coated with an antibody against human GIP (1-42) active form in which samples are added to the wells. After incubation and plate washing, HRP labeled antibody solution is added to form an antibody-antigen labeled antibody complex. Finally, HRP enzyme activity is determined by TMB and the concentration of active GIP is calculated.

D. Kit Storage

1. Upon receipt of the Active GIP ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (96 wells)	1 pack
STD	Standard	1 vial
HRPAS	HRP Labeled Antibody Solution	1 x 12 mL
TMB	Enzyme Substrate Solution (TMB)	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
BUF	Buffer Solution	1 x 25 mL
WASH	Washing Solution (20X Concentrated)	1 x 50 mL
SEAL	Adhesive seal	3 sheets

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled water
Polypropylene microtubes
Volumetric flasks
Microplate shaker
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (50 µL), pipetting should be done as carefully as possible. A high quality 100 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

An EDTA-2Na (1 mg/mL) additive blood collection tube is recommended for plasma collection. After blood is collected, add DPP-4 inhibitor (0.01 mL per mL of blood) to the collection tube immediately. Plasma samples should be used as soon as possible after collection. For later testing, samples should be aliquoted and stored at below -70°C to ensure maximum stability. Avoid repeated freezing and thawing.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use.
2. Standard
   The standard is provided in lyophilized form (500 pg/vial). Working standards should be prepared immediately prior to use as described in Section I.2. The working standard concentrations are 0, 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 pM. To prepare working standards, the lyophilized standard must be first reconstituted. The reconstituted 100 pM (500 pg/mL) standard once prepared is recommended to be stored frozen at below -70°C if not used all at once. The reconstituted standard should be not be repeatedly thawed, so the 100 pM standard should be appropriately aliquoted in appropriate volumes prior to being frozen. The reconstituted standard is stable for at least 2 months at below -70°C. The working standards should be discarded after each use.

3. HRP Labeled Antibody Solution
   Provided as ready to use.
4. Enzyme Substrate Solution (TMB)
   Provided as ready to use.
5. Stop Solution
   Provided as ready to use.
6. Buffer Solution
   Provided as ready to use.
7. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water.

I.2. Preparation of working standards

1. Reconstitute standard (500 pg/vial) with 1 mL of Buffer Solution (marked "BUF") and mix thoroughly, resulting in a 100 pM (500 pg/mL) reconstituted standard.
2. Dispense 0.2 mL of Buffer Solution into seven polypropylene microtubes labeled 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 pM.
3. Dispense 0.2 mL of the 100 pM standard into the 50 pM microtube, and mix thoroughly.
4. Dispense 0.2 mL of the 50 pM standard into the 25 pM microtube, and mix thoroughly.
5. Dispense 0.2 mL of the 25 pM standard into the 12.5 pM microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 0.2 mL of Buffer Solution into one polypropylene microtube labeled 0 pM. You should now have working standards of 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 pM.*

* For samples anticipated to have a very low concentration, a further dilution to create a 0.39 pM working standard is possible.

Please note: Working standards should be prepared immediately prior to use.

I.3. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. To maximize stability, reconstituted standard should be stored at below -70°C. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Aspirate well contents and wash three times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
2. In each well, add 50 µL of Buffer Solution.
3. In each well, add 50 µL of sample or standard and mix well by repeated pipetting.
4. Cover the wells with adhesive seal and incubate the plate for 2 hours at room temperature on a microplate shaker (shake at 100 rpm).
5. Aspirate well contents and wash four times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
6. Add 100 µL of the HRP Labeled Antibody Solution in each well.
7. Cover the wells with adhesive seal and incubate the plate for 1 hour at room temperature on a microplate shaker (shake at 100 rpm).

8. Aspirate well contents and wash four times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
9. Add 100 µL of Enzyme Substrate Solution (TMB) in each well. Cover the wells with adhesive seal and incubate the plate for 30 mins in dark room at room temperature without shaking.
10. Stop the reaction by adding 100 µL of Stop Solution to each well.
11. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the active GIP concentration

1. Using computer software, construct the active GIP calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding active GIP concentration on the X axis. A higher-grade polynomial or four parametric logistic (4-PL) curve fit are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Active GIP concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The active GIP concentration is expressed in pM.
   Note: *Samples with high active GIP concentrations (ie. fall above the range of the assay) should be further diluted with the Buffer Solution and rerun.*

J. Performance characteristics

J.1. Assay range

The Active GIP ELISA Kit has an assay range from 0.78 pM – 50 pM (3.9 – 250 pg/mL).

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

J.3. Cross reactivity

The assay has a high specificity for GIP (1-42) active form and shows no cross reactivity to GIP (3-42) inactive form, glucagon, GLP-2, GLP-1 (7-36) NH_2, and GLP-1 (9-36) NH_2.

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