KAMIYA BIOMEDICAL COMPANY

# Mouse Total GIP ELISA

**For the quantitative determination of mouse total GIP in plasma and culture medium supernatant**

Cat. No. KT-1890

**For Research Use Only.**

## PRODUCT INFORMATION

# Mouse Total GIP ELISA
**Cat. No. KT-1890**

## INTENDED USE
The Mouse Total GIP ELISA is for the quantitative determination of mouse total GIP in plasma and culture medium supernatant. For research use only.

## INTRODUCTION
The incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagons-like peptide-1 (GLP-1), are 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.
The intestinal peptide GIP was first isolated from pig upper small intestine. The sequences of pig, bovine and human GIP have been determined, each has 42 amino acids, and the sequences are highly conserved. The pig and bovine peptides differ from the human at two and three sites, respectively. Researchers have isolated a human cDNA encoding the GIP precursor and confirming that GIP belongs to the vasoactive intestinal peptide (VIP)/Glucagon/secretin family. GIP is a gastrointestinal peptide hormone that 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. Plasma level of GIP is elevated after an oral glucose load or a meal in normal humans. This increase after a meal is below normal in newly diagnosed insulin–dependent diabetics. It is now being recognized that GIP receptor is also expressed in organs and cells such as duodenum, small intestine, pancreatic alpha-cell, adipocyte and osteoblast. These results demonstrate GIP may have a lot of physiological effects in addition to their glucoregulatory effects.
GIP is rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4) to GIP (3-42) with a blood half-life of only several minutes. DPP-4 inhibitor can prolong the half-life of GIP, that expecting treatment of incretin effect.
The kit can be used for measurement of total GIP [both GIP (1-42) and GIP (3-42)] in mouse plasma with high sensitivity. It will be a specifically useful tool for incretin research.

## PRINCIPLE
This ELISA kit for determination of mouse total GIP is based on a sandwich enzyme immunoassay with two monoclonal antibodies. Calibrators or samples, and HRP labeled antibodies are added to the wells of plate coated with antibodies against mouse GIP. During the incubation antibody–antigen–labeled antibody complex is formed on the surface of the wells. After the incubation and rinsing out excess labeled antibody, HRP enzyme activity is finally determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of GIP is calculated.
The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influence by other components in samples. GIP calibrator is highly purified synthetic product.
This ELISA kit has high specificity to mouse GIP (1-42) and GIP (3-42), and shows no cross reactivity to Glucagon, GLP-1 (7-37), GLP-1 (7-36) $NH_2$, GLP-1 (9-36) $NH_2$ and mouse GLP-2.

## COMPONENTS

| Component | Form | Quantity | Main Ingredient |
|---|---|---|---|
| 1. Antibody-Coated Plate | Microtiter plate | 1 plate (96-well) | Mouse anti-GIP monoclonal antibody coated |
| 2. Calibrator | Lyophilized | 1 vial (1.2 pmol) | Synthetic mouse GIP |
| 3. HRP Labeled Antibody | Liquid | 1 bottle (6 mL) | HRP labeled mouse anti-GIP monoclonal |
| 4. Enzyme Substrate (TMB) | Liquid | 1 bottle (12 mL) | 3,3',5,5'-Tetramethylbenzidine (TMB) |
| 5. Stopping Solution | Liquid | 1 bottle (12 mL) | 1M Sulfuric acid |
| 6. Buffer Solution | Liquid | 1 bottle (12 mL) | Buffer containing a reaction accelerator |
| 7. Concentrated Wash Solution | Liquid | 1 bottle (50 mL) | Concentrated saline |
| 8. Plate Seal | | 2 sheets | |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 3.0 at 450 nm
- Washing device for microtiter plate and dispenser with aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Glass test tubes for preparation of calibrator solution
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

## PRECAUTIONS

1. EDTA-2Na additive blood collection tube is recommended for the plasma collection. Plasma samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30℃. Avoid repeated freezing and thawing of samples.

2. Calibrator solutions should be prepared immediately before use. If the kit is used dividedly, the rest of the reconstituted calibrator solution be stored at or below -30℃ and used within 2 months.

3. As pipetting operations may affect precision of the assay, pipette calibrator solutions or samples precisely into the wells of assay plate. In addition, use clean test tubes or vessels in assay and a new tip for each calibrator diluting process and for each sample to avoid cross contamination.

4. Perform all the determination in duplicate.

5. To quantitate accurately, always run a calibration curve for each assay.

6. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

7. Protect the reagents from strong light (e.g. direct sunlight) during storage and assay.

8. Satisfactory performance of the assay will be guaranteed only when reagents are used from combination pack with identical lot number.

9. During storage of washing solution (concentrated) at 4℃, precipitates may be observed, however, they will be dissolved when diluted.

10. When sample concentration exceeds 600 pM, it needs to be diluted with buffer solution to proper concentration.

## REAGENT PREPARATION

1. Preparation of calibrator solution: Reconstitute lyophilized Calibrator with 1 mL of buffer solution, which affords 1.2 pmol/mL (1,200 pM) stock solution. The reconstituted stock solution (0.2 mL) is diluted with 0.2 mL of buffer solution that yields 600 pM calibrator solution. Then 0.1 mL of 600 pM calibrator solution is diluted with 0.2 mL of buffer solution that yields 200 pM calibrator solution. Repeat the dilution procedure to make each calibrator solution of 66.7, 22.2, 7.4 and 2.5 pM. Buffer solution is used as 0 pM calibrator solution.

2. Dilution of Wash Solution Concentrate: Dilute Wash Solution Concentrated (50 mL) to 1,000 mL with deionized or distilled water.

3. Other reagents are ready for use.

## STORAGE

Store kit at 4°C.

## ASSAY PROTOCOL

1. Before starting assay, bring all the reagents and test samples to room temperature (20-30℃).

2. Fill 0.35 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

3. Add 10 µL of each of calibrator solutions (0, 2.5, 7.4, 22.2, 66.7, 200 and 600 pM) or samples to the wells first, and then 50 µL of HRP labeled antibody solution to each of the wells.

4. Cover the plate with adhesive foil and incubate it at 4℃ for 18-20 hours.

5. After incubation, take off the adhesive foil, aspirate and wash the wells 6 times with 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

6. Add 100 µL of Enzyme substrate solution (TMB) to each of the wells, cover the plate with adhesive foil and keep it for 30 minutes at room temperature in a dark place for color reaction (keep still, plate shaker not needed).

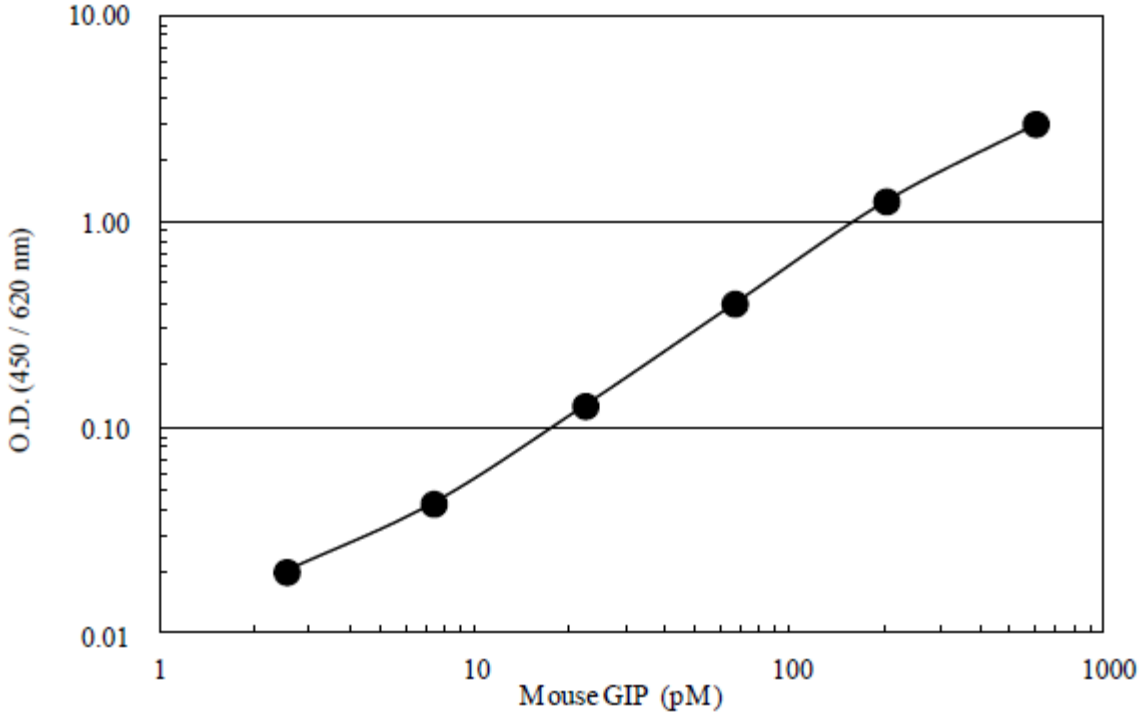
7. Add 100 µL of Reaction Stopping Solution into each well to stop color reaction.

8. Read the optical absorbance of the solution in the wells at 450 nm. The dose-response curve of this assay fits best to a 5 (or 4)-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5 (or 4)-parameter logistic function.
Otherwise calculate mean absorbance values of wells containing calibrators and plot a calibration curve on double logarithmic graph paper (abscissa: concentration of calibrator; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this calibration curve.

## PERFORMANCE

**Assay Range**
2,5 – 600 pM

**Typical Calibration Curve** (example only, a new calibration curve for each run must be established by the end-user)




**Precision and Reproducibility**

| Test sample | Intra-assay CV (%) | Inter-assay CV (%) |
|---|---|---|
| Mouse plasma | 2.1~ 5.4 | 2.9~ 6.2 |

## Analytical Recovery

<Mouse plasma A>

| Added GIP (pM) | Observed (pM) | Expected (pM) | Recovery (%) |
|---|---|---|---|
| 0 | 67.5 | | |
| 10 | 75.7 | 77.5 | 97.7 |
| 50 | 119.1 | 117.5 | 101.3 |
| 100 | 162.8 | 167.5 | 97.2 |

< Mouse plasma B>

| Added GIP (pM) | Observed (pM) | Expected (pM) | Recovery (%) |
|---|---|---|---|
| 0 | 63.6 | | |
| 10 | 77.1 | 73.6 | 104.8 |
| 50 | 124.2 | 113.6 | 109.4 |
| 100 | 190.5 | 163.6 | 116.5 |

< Mouse plasma C>

| Added GIP (pM) | Observed (pM) | Expected (pM) | Recovery (%) |
|---|---|---|---|
| 0 | 124.1 | | |
| 10 | 135.9 | 134.1 | 101.3 |
| 50 | 183.2 | 174.1 | 105.2 |
| 100 | 252.3 | 224.1 | 112.6 |

## Dilution Test

< Mouse plasma A>

| Sample dilution | Observed (pM) | Expected (pM) | % of Expected (%) |
|---|---|---|---|
| X1 | 166.4 | 166.4 | |
| X2 | 86.7 | 83.2 | 104.3 |
| X4 | 38.5 | 41.6 | 92.6 |
| X8 | 16.8 | 20.8 | 80.7 |

< Mouse plasma B>

| Sample dilution | Observed (pM) | Expected (pM) | % of Expected (%) |
|---|---|---|---|
| X1 | 121.8 | 121.8 | |
| X2 | 63.7 | 60.9 | 104.7 |
| X4 | 29.3 | 30.5 | 96.2 |
| X8 | 12.5 | 15.2 | 81.8 |

< Mouse plasma C>

| Sample dilution | Observed (pM) | Expected (pM) | % of Expected (%) |
|---|---|---|---|
| X1 | 136.5 | 136.5 | |
| X2 | 73.0 | 68.2 | 106.9 |
| X4 | 34.1 | 34.1 | 100.0 |
| X8 | 14.8 | 17.1 | 87.0 |

**FOR RESEARCH USE ONLY**

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com