Mouse Total GIP ELISA

For the quantitative determination of total glucose-dependent insulinotropic polypeptide in mouse plasma.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 48-GIPMS-E01
Size: 96 wells
Version: January 27, 2020 – ALPCO 1.1

Intended Use

The kit can be used for measurement of total GIP [both GIP (1-42) and GIP (3-42)] in mouse plasma with high sensitivity. For Research Use Only. Not for Use in Diagnostic Procedures.

Introduction

The incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-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 porcine upper small intestine[1]. The sequences of porcine[2,3], bovine[4] and human GIP[5] have been determined, each have highly conserved sequences of 42 amino acids. The porcine and bovine peptides differ from the human at two and three sites, respectively. Takeda et al. have isolated a human cDNA encoding the GIP precursor and confirming that GIP belongs to the vasoactive intestinal peptide (VIP)/Glucagon/secretin family[6]. GIP is a gastrointestinal peptide hormone that is released from duodenal endocrine K cells after absorption of glucose or fat[7]. GIP is a potent releaser of insulin in experimental animals[8] and in humans [9,10] provided that the blood glucose is above basal level. Plasma level of GIP is elevated after an oral glucose load or a meal in human subjects. This increase after a meal is below normal in newly diagnosed insulin–dependent diabetics[11]. It is now being recognized that GIP receptor is also expressed in organs and cells such as the duodenum, small intestines, pancreatic alpha-cells, adipocytes and osteoblasts. These results demonstrate GIP may have other physiological effects in addition to their glucoregulatory effects[12,13,14,15]. 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 inhibitors can prolong the half-life of GIP.

GIP ELISA Performance Summary

- The assay kit can measure total GIP in plasma within the range of 2.5~ 600 pM.
- The assay is completed within 18 ~ 20hr + 0.5hr.
- With one assay kit, 41 samples can be measured in duplicate.
- Test sample: Mouse plasma (EDTA-2Na)
- Sample volume: 10µL
- The 96-well plate of this kit consists of 8-wells strips that can be used separately.
- Stability and storage:
    - Store all components at 2-8°C.
    - This kit is stable under this condition for 24 months from the date of manufacture.
    - The expiry date is stated on the package.

Contents Summary

1. Antibody coated plate
2. GIP standard
3. HRP labeled antibody solution
4. Enzyme substrate solution (TMB)
5. Stop Solution
6. Buffer Solution
7. Wash Solution Concentrate
8. Adhesive foil

Assay Characteristics

This ELISA kit is used for quantitative determination of mouse total GIP in plasma. This kit is characterized by its sensitive quantification and high specificity. In addition, it has no influence by other components in samples. The GIP standard is a highly purified synthetic product.

Analytical Specificity

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.

Principle of the Assay

This ELISA kit for determination of mouse total GIP is based on a sandwich enzyme immunoassay with two monoclonal antibodies. Standards 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 using 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of GIP is calculated.

Materials

Component	Form	Quantity	Main Ingredient
Antibody coated plate	Microtiter plate	1 plate x 96 wells	Mouse anti-GIP monoclonal antibody coated
Standard	Lyophilized	1 vial x 1.2 pmol	Synthetic mouse GIP
HRP labeled antibody solution	Liquid	1 bottle (6 mL)	HRP-labeled mouse anti GIP monoclonal antibody
Enzyme Substrate Solution (TMB)	Liquid	1 bottle x 12 mL	3,3',5,5'-Tetramethylbenzidine (TMB)
Stop Solution	Liquid	1 bottle x 12 mL	1M H_2SO_4
Buffer Solution	Liquid	1 bottle x 12 mL	Buffer containing a reaction accelerator
Wash Buffer	Liquid	1 bottle x (50 mL)	Concentrated Saline
Adhesive Foil	-	2 pieces	-

Equipment Required, but Not Provided

1. Microtiter plate reader which can read extinction 3.0 at 450 nm
2. Washing device for microtiter plate and dispenser with aspiration system
3. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
4. Test tubes for preparation of standard solution
5. Graduated cylinder (1000 mL)
6. Distilled water or deionized water
7. Timer

Sample Handling

Preparation of plasma samples:
EDTA-2Na additive blood collection tube is recommended for the plasma collection. Alternatively, BDTM P800 Venous Blood Collection Tubes for plasma GLP-1, GIP, Glucagon, Ghrelin (Becton, Dickinson) can be used. 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°C. Avoid repeated freezing and thawing of samples.

Reagent Preparation

Preparation of standard solution:
Standard solutions should be prepared immediately before use. Well strips can be used separately. In such case, the rest of the reconstituted reagent (standard) should be stored at or below -30°C（stable for 2 months）

Reconstitute the mouse GIP standard with 1 mL of buffer solution, which affords 1.2 pmol/mL (1200 pM) standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.2 mL of buffer solution that yields 600 pM standard solution. The 600 pM standard solution (0.1 mL) is diluted with 0.2 mL of buffer solution that yields 200 pM standard solution. Repeat the dilution procedure to make each standard solution of 66.7, 22.2, 7.4 and 2.5 pM. Buffer solution itself is used as the 0 pM standard solution.

Preparation of washing solution:
Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled or deionized water. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however, they will be dissolved when diluted.

Other reagents are ready for use.

Assay Procedures

Standards and samples should be tested in duplicate.

1. Before starting the assay, bring all the reagents and samples to room temperature (20 ~ 30ºC).

2. Fill 0.35 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure 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 10µL of each of standard 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 at 2 ~ 8ºC for 18 ~ 20 hours (shaker not needed).

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

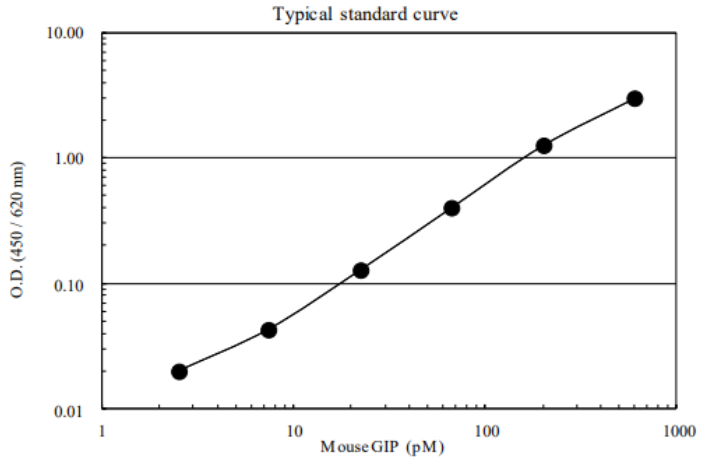
7. Add 100 µL of stopping solution to each of the wells to stop color reaction.

8. Read the optical absorbance of the solution in the wells at 450 nm as soon as possible after stopping the color reaction.

Calculation of Results

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 standards and plot a standard curve on double logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Performance Characteristics



Typical standard curve. The curve shown here should not be used for calculation.

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)	Recovery %
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)	Recovery %
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)	Recovery %
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

Crossreactivity

Related peptides	(%) Crossreactivity
GIP (Mouse)	100
Glucagon	<0.1
GLP-1 (7-37)	<0.1
GLP-1 (7-36) NH_2	<0.1
GLP-1 (9-36) NH_2	<0.1
Mouse GLP-2	<0.1

Precision and Reproducibility

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

Assay range

2.5 ~ 600 pM

Stability and Storage

Storage
Store all of the components at 2-8°C.

Shelf life
The kit is stable under this condition for 24 months from the date of manufacturing. The expiry date is stated on the label of the kit.

Package
For 96 tests per one kit including standards.

References

1. Brown,J.C., Mutt, V. and Pedersen,R.A. (1970) Further purification of a polypeptide demonstrating enterogastrone activity. J.Physiol. 209, 57-64
2. Jörnvall H, Carlquist M, Kwauk S, Otte SC, McIntosh CH, Brown JC, Mutt V. (1981) Amino acid sequence and heterogeneity of gastric inhibitory polypeptide (GIP). FEBS Lett. 123, 205-210.
3. Moody,A.J., Damm Jorgensen, K.and Thim, L.(1981)Diabetologia 21, 306, abstr.
4. Carlquist M, Maletti M, Jörnvall H, Mutt V. (1984) A novel form of gastric inhibitory polypeptide (GIP) isolated from bovine intestine using a radioreceptor assay. Fragmentation with staphylococcal protease results in GIP1-3 and GIP4-42, fragmentation with enterokinase in GIP1-16 and GIP17-42. Eur.J. Biochem. 145, 573-577

5. Moody, A. J., Thim, L. & Valverde, I. (1984) The isolation and sequencing of human gastric inhibitory peptide(GIP). FEBS Lett. 172, 142-148
6. Takeda J, Seino Y, Tanaka K, Fukumoto H, Kayano T, Takahashi H, Mitani T, Kurono M, Suzuki T, Tobe T, et al.(1987) Sequence of an intestinal cDNA encoding human gastric inhibitory polypeptide precursor. Proc Natl Acad Sci U S A. 84(20):7005-8.
7. Pederson, R.A. (1994) in Gut Peptides: Biochemistry and Physiology, eds, Walsh, J.H.& Dockray, G.J. (Raven, New York), pp,217-260
8. Rabinovitch, A. and Dupre, J (1974)Effect of the gastric inhibitory polypeptide present in impure pancreozymin-cholecystokinin of plasma insulin and glucagons in the rat. Endocrinology 94,1139-1144
9. Dupre,J., Ross, S.A.,Watson, D. and Brown, J.C. (1973) Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J. Clin. Endocrinol. Metab. 37, 826-828
10. Elahi, D., Andersen, D.K., Brown, J.C.,Debas, H.T., Hershcopf, R.J., Raizes, G.S., Tobin, J.D. and Andres, R.(1979) Pancreatic alpha-and-beta-cell response to GIP infusion in normal man. Am.J.Physiol. 237, E185-E191
11. Krarup T, Madsbad S, Moody AJ, Regeur L, Faber OK, Holst JJ, Sestoft L.(1983) Diminished immunoreactive gastric inhibitory polypeptide response to a meal in newly diagnosed type I (insulin-dependent) diabetics. J Clin Endocrinol Metab. 56, 1306-12.
12. Naitoh R, Miyawaki K, Harada N, Mizunoya W, Toyoda K, Fushiki T, Yamada Y, Seino Y, Inagaki N.(2008) Inhibition of GIP signaling modulates adiponectin levels under high-fat diet in mice. Biochem Biophys Res Commun. 376, 21-5.
13. Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J, Seino Y. (1999) Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. Proc Natl Acad Sci U S A. 96, 14843-7.
14. Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, Hiai H, Mizunoya W, Fushiki T, Holst JJ, Makino M, Tashita A, Kobara Y, Tsubamoto Y, Jinnouchi T, Jomori T, Seino Y.(2002) Inhibition of gastric inhibitory polypeptide signaling prevents obesity. Nat Med. 8, 738-42.
15. Tsukiyama K, Yamada Y, Yamada C, Harada N, Kawasaki Y, Ogura M, Bessho K, Li M, Amizuka N, Sato M, Udagawa N, Takahashi N, Tanaka K, Oiso Y, Seino Y. (2006) Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion. Mol Endocrinol. 20, 1644-51.