YK256 Mouse GIP (Total)-HS ELISA

人と科学のステキな未来へ

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Contents

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– Please read all the package insert carefully before beginning the assay –

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Ⅰ. 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 porcine upper small intestine[1]. The sequences of porcine[2][3], bovine[4] and human GIP[5] have been determined, each has 42 amino acids, and the sequences is highly conserved. The porcine and bovine peptides differ from the human at two and three site, 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 man [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 normal man. 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 duodenum, small intestine, pancreatic alpha-cell, adipocyte and osteoblast. These results demonstrate GIP may have a lot of physiological effect 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 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.

YK256 Mouse GIP (Total)-HS ELISA Kit	**Contents**
▼ The assay kit can measure total GIP in plasma within the range of 2.5~ 600 pM.	1) Antibody coated plate 2) Standard
▼ The assay is completed within 18 ~ 20hr + 0.5hr.	3) HRP labeled antibody solution
▼ With one assay kit, 41 samples can be measured in duplicate.	4) Enzyme substrate solution (TMB)
▼ Test sample: Mouse plasma (EDTA-2Na) and culture medium supernatant Sample volume: 10 μL	5) Stopping solution 6) Buffer solution 7) Washing solution (concentrated)
▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.	8) Adhesive foil
▼ Stability and storage Store all of the components at 2-8℃. The kit is stable under the condition for 24 months from the date of manufacturing. The expiry date is stated on the label of kit.	

Ⅱ. Characteristics

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

< 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.

< Assay principle >
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 by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of GIP is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Mouse anti GIP monoclonal antibody coated
2.	Standard	lyophilized	1 vial (1.2 pmol)	Synthetic mouse GIP
3.	HRP labeled antibody solution	liquid	1 bottle (6 mL)	HRP labeled mouse anti GIP monoclonal antibody
4.	Enzyme substrate solution (TMB)	liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
5.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
6.	Buffer solution	liquid	1 bottle (12 mL)	Buffer containing a reaction accelerator
7.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
8.	Adhesive foil		2 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader) which can read extinction 3.0 at 450nm

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. Glass test tubes for preparation of standard solution

5. Graduated cylinder (1,000 mL)

6. Distilled water or deionized water

< Preparatory work >

1. Preparation of standard solution:
   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 0 pM standard solution.

2. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled or deionized water.

3. Other reagents are ready for use.

< Procedure >

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 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 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 it at 2 ~ 8ºC for 18 ~ 20 hours (keep still, plate shaker not need).

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 of the well, 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 need).

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. 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.

V. Notes

1. EDTA-2Na additive blood collection tube is recommended for the plasma collection. If same blood sample is to be prepared for measuring GIP (1-42) active form only using YK252 Mouse GIP (Active) ELISA kit [this YK256 kit can measure both of GIP (1-42) and GIP (3-42)], DPP-4 inhibitor should be added immediately after blood is collected (0.01mL per milliliter of blood, Catalog No. DPP4 MILLIPORE) to collection tube immediately. Alternatively BD^{TM} 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.

2. Standard solutions should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagent (standard) should be stored at or below -30°C（stable for 2 months）.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however, they will be dissolved when diluted.

4. Pipetting operations may affect the precision of the assay, so that pipette standard solutions or samples precisely into each well of plate. In addition, use clean test tubes or vessels in assay and use new tip for each standard or sample to avoid cross contamination.

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

6. Perform all the determination in duplicate.

7. Read plate optical absorbance of reaction solution in wells as soon as possible after stop color reaction.

8. To quantitate accurately, always run a standard curve when testing samples.

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

10. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

Ⅵ. Performance Characteristics

Typical standard curve

<Analytical Recovery>

<Mouse plasma A>
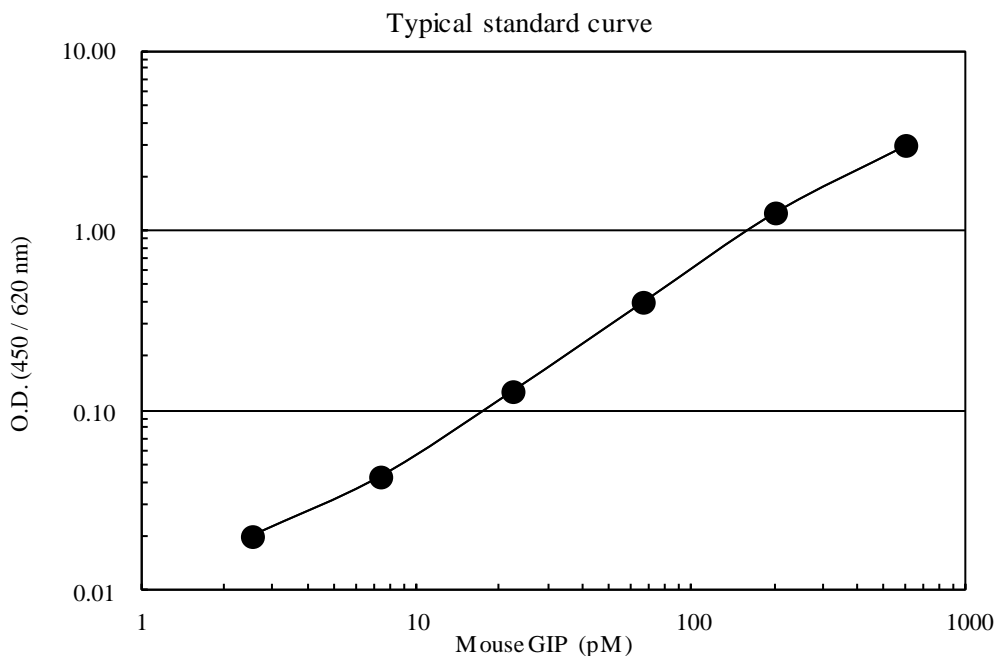
Added GIP (pM)	Observed (pM)	Expected (pM)	Recovery (%)
0	67.5		
10	75.7	77.5	97.7
50	119.1	117.5	101.3
100	162.8	167.5	97.2

< 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

<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

Ⅶ. Stability and Storage

< Storage >	Store all of the components at 2-8°C.
< Shelf life >	The kit is stable under the condition for 24 months from the date of manufacturing. The expiry date is stated on the label of kit.
< Package >	For 96 tests per one kit including standards

Ⅷ. References

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