COSMO BIO CO., LTD.
Inspiration for Life Science

YK252 Mouse GIP (Active) ELISA

FOR LABORATORY USE ONLY

YANAIHARA INSTITUTE INC.

Contents

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

YK252 Mouse GIP (Active) ELISA

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.

This ELISA kit has high specificity to mouse GIP (1-42) active form and shows no crossreactivity to mouse GIP (3-42) inactive form.

YK252 Mouse GIP (Active) ELISA Kit

▼ The assay kit can measure mouse GIP (1-42) active form in plasma within the range of 7.8 ~ 500 pg/mL. (1.56～99.9 pM)

▼ The assay is completed within 2hr+1hr+0.5hr.

▼ With one assay kit, 40 samples can be measured in duplicate.

▼ Test sample: mouse plasma (EDTA-2Na + DPP-4 inhibitor) and culture medium supernatant
Sample volume: 25 μL or 15μL

▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.

▼ Stability and storage
Store all of the components at 2-8ºC.
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.

Contents

1) Antibody coated plate
2) Standard
3) HRP labeled antibody solution
4) Enzyme substrate solution (TMB)
5) Stopping solution
6) Buffer solution
7) Washing solution (concentrated)
8) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of mouse GIP (1-42) active form 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 (1-42) standard is highly purified synthetic product.

< Specificity >

This ELISA kit has high specificity to mouse GIP (1-42) active form and shows no cross reactivity to mouse GIP (3-42) inactive form, glucagons, mouse GLP-2, GLP-1 (7-36) NH_2 and GLP-1 (9-36) NH_2.

< Assay principle >

This ELISA kit for determination of mouse GIP (1-42) active form is based on a sandwich enzyme immunoassay. To the wells of plate coated with highly purified mouse monoclonal antibody against mouse GIP (1-42) active form, standards or samples are added for the 1st step immunoreaction. After the 1st step incubation and plate washing, HRP labeled antibody solution against mouse GIP (1-42) is added as the 2nd step to form antibody - antigen - labeled antibody complex on the surface of the wells. After the 2nd step incubation and rinsing out excess labeled antibody, Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of mouse GIP (1-42) active form is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Mouse anti GIP (1-42) monoclonal antibody coated
2.	Standard	lyophilized	1 vial (1000 pg)	Synthetic mouse GIP (1-42)
3.	HRP labeled antibody solution	liquid	1 bottle (12 mL)	HRP labeled mouse anti GIP (1-42) monoclonal antibody
4.	Enzyme substrate solution	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 (25 mL)	Buffer containing a reaction accelerator
7.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
8.	Adhesive foil		3 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader) which can read extinction 3.0 at 450nm
2. Microtiter plate shaker
3. Washing device for microtiter plate and dispenser with aspiration system
4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
5. Glass test tubes for preparation of standard solution
6. Graduated cylinder (1,000 mL)
7. Distilled water or deionized water

< Preparatory work >

1. Preparation of standard solution:
   Reconstitute the mouse GIP (1-42) standard with 1 mL of buffer solution, which affords 1000 pg/mL standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.2 mL of buffer solution that yields 500 pg/mL standard solution. Repeat the dilution procedure to make each standard solution of 250, 125, 62.5, 31.3, 15.6 and 7.8 pg/mL. Buffer solution itself is used as 0 pg/mL standard solution. If a sample concentration below 7.8 pg/mL is predicted, standard curve may be further set up a lower detection limit by using 3.9 pg/mL standard solution which can be prepared by 2-fold dilution of 7.8 pg/mL 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 25µL of buffer solution to the wells first, and then introduce 25µL of each of standard solutions (0, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 pg/mL) or samples to the wells.
   Sample volume can be reduced to 15µL. In such case, the volume of buffer solution to be added also reduces to 15µL. There is no change in the following procedure. Almost the same performance characteristics will be obtained even in the case of sample volume 15µL.

4. Cover the plate with adhesive foil and incubate it at room temperature for 2 hours. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).

5. After incubation, take off the adhesive foil, aspirate and wash the wells 4 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 HRP labeled antibody solution to each of the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).

8. Take off the adhesive foil, aspirate and wash the wells 4 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.

9. Add 100µL 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 need).

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

11. 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. After blood is collected, add DPP-4 inhibitor (0.01mL per milliliter of blood, Catalog No. DPP4 MILLIPORE) to collection tube immediately. Alternatively BD™ 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 500 pg/mL, it needs to be diluted with buffer solution to proper concentration.

6. During the incubation except the color reaction, the plate should be shaken gently by a plate shaker to promote immunoreaction (approximately 100 rpm).

7. Perform all the determination in duplicate.

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

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

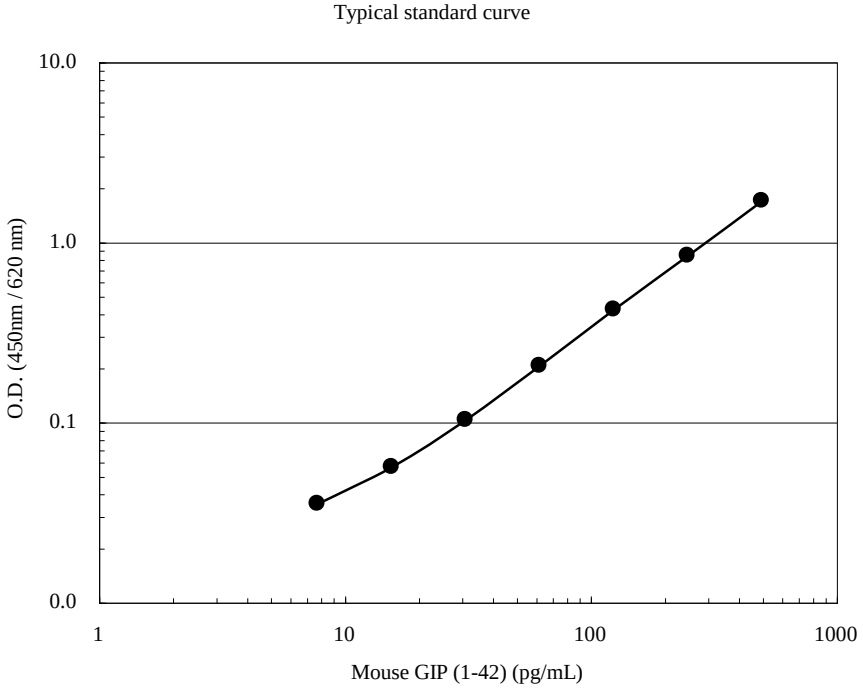
10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

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

12. Some reagents contain human serum (tested and found negative for HBsAG, HIV 1/2, HCV, HIV-1 AG or HIV-1 NAT, ALT and a test for Syphilis by FDA approved methods), care should be taken when handling.

13. Few floating matter may be rarely observed in buffer solution. It does not affect to the performance of measurement. After bring to room temperature, please agitate lightly before use.

Ⅵ. Performance Characteristics



<Analytical Recovery>

<Mouse plasma A>

Added GIP (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0	16.3		
10	24.4	26.3	92.8
50	60.8	66.3	91.7
200	230.7	216.3	106.7

<Mouse plasma B>

Added GIP (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0	34.3		
10	44.5	44.3	100.5
50	82.0	84.3	97.3
200	241.0	234.3	102.9

<Mouse plasma C>

Added GIP (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0	20.8		
10	28.8	30.8	93.5
50	65.4	70.8	92.4
200	225.5	220.8	102.1

<Mouse plasma D>

Added GIP (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0	24.5		
10	34.0	34.5	98.6
50	69.2	74.5	92.9
200	223.6	224.5	99.6

<Mouse plasma E>

Added GIP (pg/ml)	Observed (pg/ml)	Expected (pg/ml)	Recovery (%)
0	16.9		
10	28.7	26.9	106.7
50	63.5	66.9	94.9
200	209.3	216.9	96.5

<Dilution test >

<Mouse plasma A>

Sample dilution	Observed (pg/ml)	Expected (pg/ml)	% of Expected (%)
X1	26.0	26.0	
X1.5	18.3	17.3	105.4
X2	13.8	13.0	106.3
X3	9.1	8.7	105.1

<Mouse plasma B>

Sample dilution	Observed (pg/ml)	Expected (pg/ml)	% of Expected (%)
X1	30.4	30.4	
X1.5	21.7	20.3	106.8
X2	16.2	15.2	106.3
X3	10.3	10.1	101.4

<Mouse plasma C>

Sample dilution	Observed (pg/ml)	Expected (pg/ml)	% of Expected (%)
X1	29.0	29.0	
X1.5	19.8	19.3	102.6
X2	15.3	14.5	105.2
X3	10.7	9.7	110.5

<Mouse plasma D>

Sample dilution	Observed (pg/ml)	Expected (pg/ml)	% of Expected (%)
X1	28.2	28.2	
X1.5	19.6	18.8	104.1
X2	15.0	14.1	106.3
X3	10.3	9.4	109.4

<Mouse plasma E>

Sample dilution	Observed (pg/ml)	Expected (pg/ml)	% of Expected (%)
X1	25.5	25.5	
X1.5	17.6	17.0	103.6
X2	13.8	12.7	108.1
X3	9.0	8.5	105.9

< GIP (1-42) concentration in mouse plasma after feeding>

Mouse	Time after feeding	1	2	Mean
No.1	**0 (fasting)**	12.1	10.3	**11.2**
	10 min.	63.4	62.2	**62.8**
	20 min.	23.5	22.0	**22.7**
No.2	**0 (fasting)**	8.4	10.3	**9.4**
	10 min.	68.4	72.5	**70.5**
	20 min.	23.0	22.5	**22.7**
No.3	**0 (fasting)**	13.3	12.1	**12.7**
	10 min.	44.0	42.3	**43.1**
	20 min.	23.0	24.5	**23.7**

pg/mL

<Crossreactivity>

Related peptides	Crossreactivity（%）
GIP (1-42) (Mouse)	100
GIP (3-42) (Mouse)	<0.1
Glucagon	<0.1
Mouse GLP-2	<0.1
GLP-1 (7-36) NH_2	<0.1
GLP-1 (9-36) NH_2	<0.1

< Precision and reproducibility >

Test sample	Intra-assay CV (%)	Inter-assay CV (%)
Mouse plasma	5.8~ 6.0	1.6 ~ 3.3

<Assay range>

7.8 ~ 500 pg/mL (1.56 pM～99.9 pM)

Ⅶ. Stability and Storage

< Storage >	Store all of the components at 2-8℃.
< 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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