YK161　Total GLP-1-HS ELISA Kit

For Measurement of Rat & Human Total GLP-1

FOR LABORATORY USE ONLY

<Distributed by>

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Contents

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

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Ⅰ. Introduction

GLP-1 is a peptide hormone from the intestinal mucosa, which is produced from its precursor, proglucagon by post transnational processing. The mammalian proglucagon [1] is synthesized in the neuroendocrine L-cell of the intestine and the alpha-cells of the pancreas. It contains within its structure the sequences of glucagon and two glucagon-like peptides (GLP-1 and GLP-2) in tandem flanked at their amino and carboxyl termini by dibasic residues. GLP-1 is a 37 amino acids peptide and produced in the small intestine and in the pancreas in the human, in either C-terminal-amidated on glycine-extended form[2) 3)].

GLP-1 (7-36) amide and its receptor are present in several brain regions and may play a role in the physiological control of feeding[4)]. Several reports have been presented as follows as to the biological activities of GLP-1. GLP-1 (7-37) and (7-36) amide is known as one of the most potent insulin secretagogues [5)].

GLP-1 (7-36) amide was supposed to improved glycemic control in patients with type 2 diabetes by increasing insulin secretion, by inhibiting glucagon secretion and by delaying gastric emptying rather than by altering extrapancreatic glucose metabolism[6)]. Intravenous GLP-1 (7-37) and (7-36) amide could normalize fasting hyperglycaemia in type 2 diabetic patients[7)]. Hyperglycaemia during parenteral nutrition could be controlled by exogenous GLP-1, whereas the chronic therapy of type 2 diabetes required GLP-1 derivatives with longer duration of action[8)]. Recombinant GLP-1 (7-36) amide was recently shown to cause significant weight loss in type 2 diabetics when administered for 6 weeks as a continuous subcutaneous infusion, 5-day treatment of hereby obese human subjects with GLP-1 at high doses by prandial subcutaneous infusion promptly slowed gastric emptying as a probable mechanism of action of increased satiety, decreased hunger and reduced food intake with an ensuing weight loss[9)].

YK161 Total GLP-1-HS ELISA Kit

- ▼ The assay kit can measure GLP-1 within the range of 1.24 – 300 pM
- ▼ The assay is completed within 18+1+0.5 and 0.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate.
- ▼ Test sample: plasma and culture medium supernatant
  Sample volume: 25 μL
- ▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%) 1.98 – 5.43
  Inter-assay CV (%) 2.21 – 3.84
- ▼ Stability and storage
  Store all of the components at 2-8°C.
  This kit is stable under the condition for 24 months from the date of manufacturing.
  The expiry date is stated on the package

Contents

1) Antibody coated plate
2) GLP-1(7-36)amide standard
3) Labeled antibody solution
4) SA-HRP solution
5) Enzyme substrate solution (TMB)
6) Stopping solution
7) Buffer solution
8) Washing solution (Concentrated)
9) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of total GLP-1 in rat and human plasma sample. The kit is characterized by sensitive quantification and high specificity. In addition, it is not influenced by other components in plasma sample and needlessness of sample pre-treatment.

<Specificity>

This ELISA kit has high specificity to GLP-1(1-36) amide, GLP-1(7-36) amide and GLP-1(9-36) amide and shows low crossreactivity to GLP-1(1-37) and GLP-1(7-37), and shows no crossreactivity to rat and human GLP-2, rat Glicentin, human Glucagon, rat mouse and human GIP (1-42), rat and human GIP (3-42).

<Assay principle>

This kit for determination of total GLP-1 in rat and human plasma and culture supernatant sample is based on the sandwich enzyme immunoassay. During first immune incubation, GLP-1(7-36)amide in standards or in samples bind to the rabbit anti GLP-1(7-36)amide antibody, which is coated on the surface of the microtiter plate. After incubation and plate washing, labeled antibody solution (biotinylated rabbit anti GLP-1(7-36)amide polyclonal antibody) is added to bind to the antibody-antigen complex. Then, HRP labeled streptoavidin (SA-HRP) is added to form antibody-antigen-biotinylated antibody complex. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of total GLP-1 is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Rabbit anti GLP-1 (7-36) amide polyclonal antibody coated
2.	Standard	lyophilized	1 vial (300 fmol)	Synthetic GLP –1 (7-36)amide
3.	Biotin labeled antibody solution	liquid	1 bottle (12 mL)	Biotinylated rabbit anti GLP -1 (7-36)amide polyclonal antibody
4.	SA-HRP solution	liquid	1 bottle (12 mL)	Horseradish peroxidase labeled streptoavidin
5.	Enzyme substrate solution	liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
6.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
7.	Buffer solution	liquid	1 bottle (20 mL)	Buffer containing a reaction accelerator
8.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
9.	Adhesive foil		4 pieces	

Ⅳ. Method

<Equipment required>

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

<Preparatory work>

1. Preparation of the standards:
   Reconstitute the GLP-1 (7-36) amide standard (lyophilized 300 fmol/vial) with 1 mL of buffer solution, which affords 300 pM standard solution. The reconstituted standard solution (0.1 mL) is diluted with 0.2 mL of buffer solution that yields 100 pM standard solution. Repeat the dilution procedure to make each standard solution of 33.3, 11.1, 3.70 and 1.24 pM standard solutions. Buffer solution itself is used as 0 pM standard solution.

2. Preparation of the washing solution:
   Dilute 50 mL of the 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 to each of 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 100 µL of buffer solution to the wells first, and then introduce 25 µL each of standard solution (0, 1.24, 3.70, 11.1, 33.3, 100, 300 pM) or samples to each of the wells.

4. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for 18 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 3 times with approximate 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 labeled antibody solution to each of the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) 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 3 times with approximate 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 100 µL of SA-HRP solution to each of the wells.

10. Cover the plate with adhesive foil and incubate the plate at room temperature (20-30°C) for 30 minutes. During the incubation, the plate should be shaken with microtiter plate shaker (approximately 100 rpm).

11. Take off the adhesive foil, aspirate and wash the wells 4 times with approximate 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.

12. 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 need).

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

14. Read the optical absorbance of 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 (1 mg/mL) 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°C. Avoid repeated freezing and thawing of samples. If same blood sample is to be prepared for measuring GLP-1 active form only using another kit, DPP-4 inhibitor should be added immediately to the blood, yielding 100 μM final concentration.

2. Standard solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted standard in glass vial or tube (300 pM) should be stored at 4°C or below –30°C is stable for 4 weeks. Diluted standard solutions should not be reused for another assay.

3. The total pipetting time of standard solutions and samples for a whole plate should not exceed 30 minutes.

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

5. 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.

6. When sample concentration exceeds 300 pM, it needs to be diluted with buffer solution to proper concentration.

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

8. Perform all the determination in duplicate.

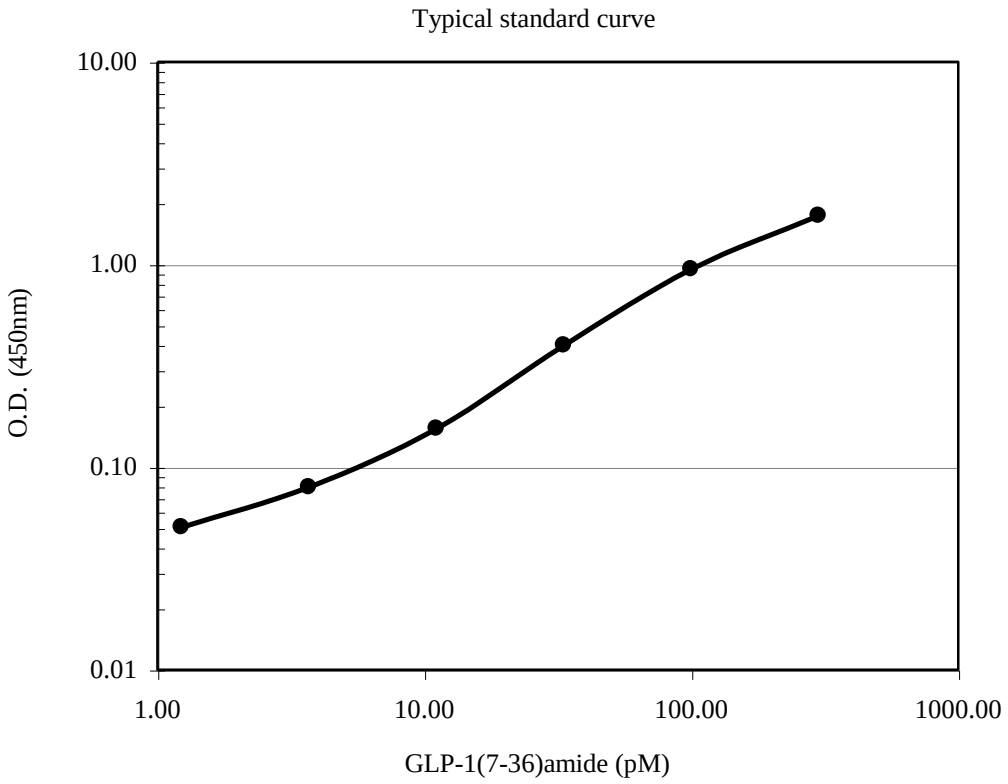
9. Read plate optical absorbance of reaction solution in wells as soon as possible after stop color reaction.

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

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

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

Ⅵ. Performance Characteristics



<Analytical recovery>

<Human Plasma A>

Added GLP-1(7-36)amide (pM)	Observed (pM)	Expected (pM)	Recovery (%)
0.00	3.41		
5.00	8.43	8.41	100.24
20.00	23.06	23.41	98.51
100.00	106.36	103.41	102.85

<Human Plasma B>

Added GLP-1(7-36)amide (pM)	Observed (pM)	Expected (pM)	Recovery (%)
0.00	4.30		
5.00	9.15	9.30	98.39
20.00	23.01	24.30	94.69
100.00	98.45	104.30	94.39

<Human Plasma C>

Added GLP-1(7-36)amide (pM)	Observed (pM)	Expected (pM)	Recovery (%)
0.00	7.99		
5.00	13.33	12.99	102.62
20.00	28.99	27.99	103.57
100.00	116.98	107.99	108.33

<Human Plasma D>

Added GLP-1(7-36)amide (pM)	Observed (pM)	Expected (pM)	Recovery (%)
0.00	21.03		
5.00	26.32	26.03	101.11
20.00	39.00	41.03	95.05
100.00	114.36	121.03	94.49

<Rat Plasma A>

Added GLP-1(7-36)amide (pM)	Observed (pM)	Expected (pM)	Recovery (%)
0.00	11.31		
5.00	14.87	16.31	91.17
20.00	26.47	31.31	84.54
100.00	82.86	111.31	74.44

<Rat Plasma B>

Added GLP-1(7-36)amide (pM)	Observed (pM)	Expected (pM)	Recovery (%)
0.00	7.91		
5.00	11.81	12.91	91.48
20.00	22.82	27.91	81.76
100.00	81.87	107.91	75.87

<Dilution test>

	Dilution ratio	Observed (pM)	Estimated (pM)	Recovery (%)
Human Plasma 1	x 1.0	27.62		
	x 2.0	15.73	31.46	113.90
	x 4.0	7.14	28.56	103.40
	x 8.0	3.08	24.64	89.21
Human Plasma 2	x 1.0	37.22		
	x 2.0	20.65	41.30	110.96
	x 4.0	10.66	42.64	114.56
	x 8.0	5.24	41.92	112.63
Human Plasma 3	x 1.0	32.07		
	x 2.0	15.78	31.56	98.41
	x 4.0	7.14	28.56	89.06
	x 8.0	3.18	25.44	79.33
Human Plasma 4	x 1.0	15.31		
	x 2.0	8.52	17.04	111.30
	x 4.0	3.96	15.84	103.46
	x 8.0	1.41	11.28	73.68

	Dilution ratio	Observed (pM)	Estimated (pM)	Recovery (%)
Rat Plasma 1	x 1.0	14.87		
	x 2.0	6.17	12.34	82.99
	x 4.0	2.89	11.56	77.74
	x 8.0	1.32	10.56	71.02
Rat Plasma 2	x 1.0	10.30		
	x 2.0	4.93	9.86	95.73
	x 4.0	2.32	9.28	90.10
	x 8.0	1.03	8.24	80.00

Precision and reproducibility

Intra-assay: Human plasma CV (%) 1.98 – 5.43

Inter-assay: Human plasma CV (%) 2.21 – 3.84

<Crossreactivity>

GLP-1 fragments	Crossreactivity(%)
GLP-1 (7-36) amide	100
GLP-1 (9-36) amide	100
GLP-1 (1-36) amide	100
GLP-1 (1-37)	9.1
GLP-1 (7-37)	9.4

GLP-1 Related peptides	Crossreactivity(%)
Rat GLP-2	<0.1
Human GLP-2	<0.1
Rat Glicentin	<0.1
Human Glucagon	<0.1
Rat GIP (1-42)	<0.1
Mouse GIP (1-42)	<0.1
Human GIP (1-42)	<0.1
Rat GIP (3-42)	<0.1
Human GIP (3-42)	<0.1

Ⅶ. Stability and Storage

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

<Shelf life> This kit is stable under the condition for 24 months from the date of manufacturing.
The expiry date is stated on the package.

<Package> For 96 tests per one kit.

Ⅷ. References

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