

YK091 Glucagon-HS ELISA

FOR LABORATORY USE ONLY

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

YK091 Glucagon-HS ELISA

I. Introduction

Glucagon is a 29-amino acid polypeptide hormone, synthesized and secreted from alpha cells of the islets of Langerhans. Glucagon generally elevates the concentration of glucose in the blood by promoting gluconeogenesis and glycogenolysis, and it has the opposite effect of insulin. The secretion of glucagon and insulin into the blood in response to the blood glucose concentration is the primary mechanism responsible for keeping the glucose levels.

This Glucagon sandwich ELISA kit has been developed by using two monoclonal antibodies specific for glucagon. This kit has high specificity to glucagon and shows no significant cross reactivity with Glicentin, Oxyntomodulin, GLP-1 and GLP-2.

YK091 Glucagon-HS ELISA Kit	Contents
▼ The assay kit can measure Glucagon within the range of 2.2 ~ 143.6 pmol/L Sensitivity : 0.3 pmol/L	1) Monoclonal antibody against glucagon coated plate
▼ The assay is completed within 18 ~ 20hr + 0.5hr.	2) Standard
▼ With one assay kit, 40 samples can be measured in duplicate.	3) HRP labeled monoclonal antibody against glucagon solution
▼ Test sample : Plasma (EDTA-2Na), serum and culture medium supernatant Sample volume : 10 µL	4) Enzyme substrate solution (TMB)
▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.	5) Stopping solution
▼ Stability and storage Store all of the components at 2-8°C. The kit is stable under the condition for 6 months from the date of manufacturing. The expiry date is stated on the label of kit.	6) Buffer solution
	7) Washing solution (concentrated)
	8) Adhesive foil

II. Characteristics

This ELISA kit is used for quantitative determination of glucagon in plasma, serum 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. Glucagon standard is highly purified synthetic product.

< Specificity >

This ELISA kit has high specificity to glucagon, and shows no significant cross reactivity to Glicentin, Oxyntomodulin, GLP-1 and GLP-2.

< Assay principle >

This ELISA kit for determination of glucagon 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 glucagon. 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 glucagon is calculated.

III. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	microtiter plate	1 plate (96 wells)	Mouse anti glucagon monoclonal antibody coated
2. Standard	lyophilized	1 vial (0.287 pmol)	Synthetic glucagon
3. HRP labeled antibody solution	liquid	1 bottle (12 mL)	HRP labeled mouse anti glucagon 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 ₂ SO 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 glucagon standard with 1 mL of buffer solution (keep still approximately five minutes and vortex well), which affords 287 pmol/L standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.2 mL of buffer solution that yields 143.6 pmol/L standard solution. Repeat the dilution procedure to make each standard solution of 71.8, 35.9, 17.9, 9.0, 4.5 and 2.2 pmol/L. Buffer solution itself is used as 0 pmol/L 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 >

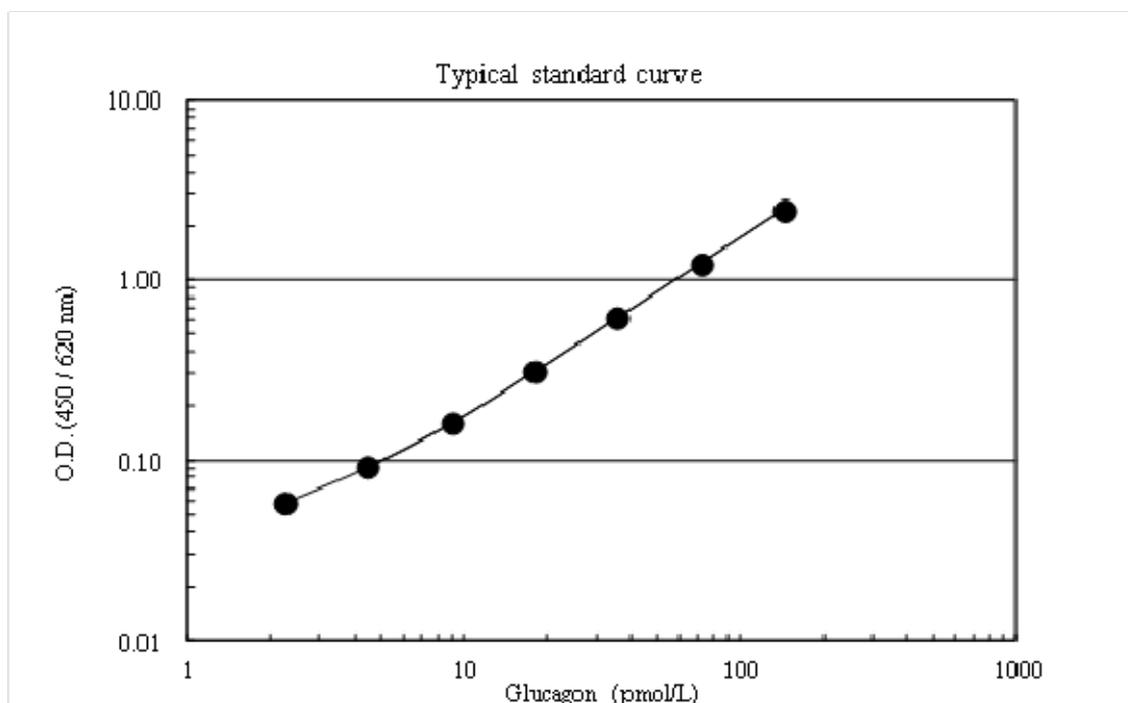
1. Before starting the assay, bring all the reagents and samples to room temperature (20 ~ 30°C).
2. Fill 0.35 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Add 10 μ L of each of standard solutions (0, 2.2, 4.5, 9.0, 17.9, 35.9, 71.8 and 143.6 pmol/L) or samples to the wells first, and then 100 μ L of HRP labeled antibody solution to each of the wells.
4. Cover the plate with adhesive foil and incubate it at 2 ~ 8°C 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 100 μ L 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 μ L 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 sample collection. If aprotinin is added, it should be added immediately after blood is collected (500KIU aprotinin per milliliter of blood). Alternatively BD™ P800 Venous Blood Collection Tubes for plasma GLP-1, GIP, Glucagon, Ghrelin (Becton, Dickinson) can be used. Plasma, serum and culture medium supernatant 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 -80°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 -80°C (stable for 1 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 143.6 pmol/L, 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.
11. Few floating matter may be rarely observed in HRP labeled antibody 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>

<Human serum A>

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	10.80		
2.9	14.05	13.67	102.8
14.4	25.67	25.16	102.0
57.4	68.39	68.22	100.2

<Human serum B>

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	4.31		
2.9	6.89	7.18	95.9
14.4	18.62	18.67	99.8
57.4	57.71	61.73	93.5

<Human plasma A>

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	12.51		
2.9	16.04	15.38	104.3
14.4	26.89	26.87	100.1
57.4	68.83	69.93	98.4

< Human plasma B >

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	10.97		
2.9	14.21	13.84	102.7
14.4	23.95	25.33	94.6
57.4	62.38	68.39	91.2

< Mouse serum A >

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	8.11		
2.9	10.92	10.98	99.4
14.4	20.48	22.47	91.2
57.4	53.75	65.53	82.0

< Mouse serum B >

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	5.60		
2.9	8.17	8.47	96.4
14.4	18.10	19.96	90.7
57.4	54.54	63.02	86.5

< Mouse plasma A >

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	18.24		
2.9	21.82	21.11	103.4
14.4	31.64	32.60	97.1
57.4	65.06	75.66	86.0

< Mouse plasma B >

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	6.55		
2.9	9.13	9.42	96.9
14.4	18.71	20.91	89.5
57.4	54.69	63.97	85.5

<Rat serum A>

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	6.46		
2.9	8.86	9.33	94.9
14.4	20.08	20.82	96.5
57.4	56.71	63.88	88.8

<Rat serum B>

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	5.46		
2.9	8.12	8.33	97.5
14.4	19.57	19.82	98.8
57.4	50.75	62.88	80.7

<Rat plasma A>

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	18.73		
2.9	22.10	21.60	102.3
14.4	34.36	33.09	103.8
57.4	69.72	76.15	91.6

<Rat plasma B>

Added Glucagon (pmol/L)	Observed (pmol/L)	Expected (pmol/L)	Recovery (%)
0	13.25		
2.9	16.32	16.12	101.2
14.4	27.61	27.61	100.0
57.4	69.51	70.67	98.4

<Dilution test >**<Human serum A>**

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	11.51	11.51	
X2	5.66	5.76	98.4
X4	2.68	2.88	93.2

<Human serum B>

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	12.14	12.14	
X2	6.23	6.07	102.6
X4	2.91	3.04	95.9

< Human plasma A >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	15.94	15.94	
X2	8.19	7.97	102.7
X4	4.11	3.99	103.1

< Human plasma B >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	14.93	14.93	
X2	7.35	7.47	98.5
X4	3.63	3.73	97.2

< Mouse serum A >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	8.60	8.60	
X2	5.01	4.30	116.5
X4	2.39	2.15	111.1
X8	0.90	1.07	83.9

< Mouse serum B >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	6.46	6.46	
X2	3.48	3.23	107.7
X4	1.53	1.62	94.7

< Mouse plasma A >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	8.38	8.38	
X2	4.22	4.19	100.9
X4	1.85	2.09	88.1

< Mouse plasma B >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	14.70	14.70	
X2	8.38	7.35	113.9
X4	4.22	3.68	114.8
X8	1.83	1.84	99.3

< Rat serum A >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	10.26	10.26	
X2	6.00	5.13	116.9
X4	2.79	2.57	108.6
X8	1.30	1.28	101.3

< Rat serum B >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	10.37	10.37	
X2	5.89	5.18	113.6
X4	2.78	2.59	107.3
X8	1.20	1.30	92.8

< Rat plasma A >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	24.69	24.69	
X2	12.26	12.35	99.3
X4	5.97	6.17	96.7
X8	2.88	3.09	93.2

< Rat plasma B >

Sample dilution	Observed (pmol/L)	Expected (pmol/L)	% of Expected (%)
X1	17.37	17.37	
X2	9.12	8.69	104.9
X4	4.34	4.34	100.0
X8	2.11	2.17	97.0

< Crossreactivity >

Related peptides	Crossreactivity (%)
Glicentin (Human)	0.68
Glicentin (Rat)	0.96
Glicentin (Mouse)	0.97
Oxyntomodulin (Human, Rat, Mouse)	0.64
Mini-glucagon	not detected
GLP-1 (7-36) NH ₂ (Human, Rat, Mouse)	not detected
GLP-1 (9-36) NH ₂ (Human, Rat, Mouse)	not detected
GLP-2 (Human)	not detected
GLP-2 (Rat)	not detected
GLP-2 (Mouse)	not detected
GIP (Human)	not detected
GIP (Rat)	not detected
GIP (Mouse)	not detected

< Precision and reproducibility >

Test sample	Intra-assay CV (%)	Inter-assay CV (%)
Serum (Human, Rat, Mouse)	1.8~ 3.5	3.5~ 9.1
Plasma (Human, Rat, Mouse)	2.1~4.6	3.4~7.1

<Assay range>

2.2 ~ 143.6 pmol/L (7.8 ~ 500 pg/mL)

<Sensitivity>

0.3 pmol/L (1.08 pg/mL)

VII. Stability and Storage

- < Storage > Store all of the components at 2-8°C.
- < Shelf life > The kit is stable under the condition for 6 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

1. Unger, R.H., Eisentraut, A.M., McCall, M.S., Keller, S., Lanz, H.C. and Madison, L.L. (1959): Glucagon antibodies and their use for immunoassay for glucagon. *Proc. Soc. Exp. Biol. Med.*, **102**: 621 - 623
2. Unger, R.H., Eisentraut, A.M., McCall, M.S. and Madison, L.L. (1961): Glucagon antibodies and an immunoassay for glucagon. *J. Clin. Invest.*, **40**: 1280-1289
3. Nishino, T., Kodaira, T., Shin, S., Imagawa, K., Shima, K., Kumahara, Y., Yanaihara, C. and Yanaihara, N. (1981): Glucagon radioimmunoassay with use of antiserum to glucagon C-terminal fragment. *Clin. Chem.*, **27**: 1690-1697
4. Jaspan, J.B., and Rubenstein, A.H. (1977): Circulating glucagon: Plasma profiles and metabolism in health and disease. *Diabetes.*, **26**: 887-902
5. Glucagon related peptides (1993): (Okuno, G., Ohneta, A. and Shima, K.ed.) pp. 52-65. Ishiyaku, Tokyo

<Manufacturer>

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