YK060　　Insulin ELISA

FOR LABORATORY USE ONLY

＜Distributed by＞

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Contents

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

YK060 Insulin ELISA Kit

Ⅰ. Introduction

This kit is a stable and convenient assay system for measurement of insulin (human, rabbit and dog) in serum samples. The processing of proinsulin, which occurs within the B cell, yields insulin and C-peptide. Insulin and C-peptide are secreted in equimolar quantities into blood circulation. Therefore, the measurement of insulin in blood is very important, and also provides valuable information to evaluate the pancreatic B cell function.

This kit for determination of insulin concentration in serum samples of human, rabbit and dog is based on a sandwich enzyme immunoassay by using combination of guinea pig anti human insulin antibody (coated on plate), recombinant insulin standard, biotinylated guinea pig anti human insulin antibody and horseradish peroxidase (HRP) labeled streptoavidin (SA). Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of insulin is calculated.

YK060 Insulin ELISA Kit

- ▼ The assay kit can measure Insulin within the range of 0.137-100 ng/mL.
- ▼ The assay is completed within 16-20 hr. + 4 hr.
- ▼ With one assay kit, 40 samples can be measured in duplicate.
- ▼ Test sample: serum (human, rabbit, dog)
  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（%） human serum 6.59- 7.10
  rabbit serum 2.51-9.08, dog serum 1.39-8.58
  Inter-assay CV（%） human serum 6.86 -11.86
- ▼ 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.

Contents

1) Antibody coated plate
2) Standard
3) Labeled antibody solution
4) SA-HRP solution
5) Substrate buffer
6) OPD tablet
7) Stopping solution
8) Buffer solution
9) Washing solution (concentrated)
10) Adhesive foil

Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of insulin in serum sample of human, rabbit and dog. The kit is characterized for sensitive quantification, high specificity and no influences with other components in samples and needlessness of sample pretreatment. Human insulin standard is recombinant product.

< Specificity >
This ELISA kit shows the following crossreactivities: 100% to human insulin, rabbit insulin and dog insulin, and 20% crossreactivity to human proinsulin.

< Assay principle >
This ELISA kit for determination of insulin is based on a sandwich enzyme immunoassay. To the wells of plate is coated with highly purified antibody against human insulin, standards or samples are added for the 1st step imunoreaction. After the 1st step incubation and plate washing, labeled antibody solution (biotinylated guinea pig anti human insulin antibody) 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, horseradish peroxidase (HRP) labeled streptoavidin (SA) is added for binding to labeled antibody. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of insulin is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Guinea pig anti human insulin antibody coated
2.	Standard	lyophilized	1 vial (100 ng)	Recombinant human insulin
3.	Labeled antibody solution	liquid	1 bottle (12mL)	Biotinylated guinea pig anti human insulin antibody
4.	SA-HRP solution	liquid	1 bottle (12mL)	Horseradish peroxidase labeled streptoavidin
5.	Substrate buffer	liquid	1 bottle (24 mL)	0.015% hydrogen peroxide
6.	OPD tablet	tablet	2 tablets	o -Phenylenediamine dihydrochloride
7.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
8.	Buffer solution	liquid	1 bottle (25 mL)	Phosphate buffer
9.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
10.	Adhesive foil		4 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (Plate reader), which can read extinction 2.5 at 490 nm (or 492nm)

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 insulin standard with 1mL of buffer solution, which affords 100 ng/mL standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.4 mL of buffer solution that yields 33.33 ng/mL standard solution. Repeat the dilution procedure to make each standard solution of 11.11, 3.70, 1.23, 0.41 and 0.137 ng/mL. Buffer solution itself is used as 0 ng/mL standard solution.
   100 ng/mL = 0.0026 IU/mL

2. Preparation of substrate solution:
   Dissolve one OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.

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

4. 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 0.35mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further three times (total 4 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 150 µL of buffer solution to the wells first, and then introduce 25 µL of each of standard solutions (0, 0.137, 0.41, 1.23, 3.70, 11.11, 33.33 and 100 ng/mL) or samples to the wells.
4. Cover the plate with adhesive foil and incubate it at 4ºC for 16~20 hours (keep still, plate shaker not need).
5. After incubation, move the plate back to room temperature keeping approximately 30 minutes and 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 100 µL of labeled antibody to each of the wells.
7. 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).
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 100 µL of SA-HRP solution to each of the wells.
10. 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).
11. Dissolve one OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.
12. 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.
13. Add 100 µL of substrate solution to each of the wells, cover the plate with adhesive foil and keep it for 30 minutes at room temperature (keep still, plate shaker not need).
14. Add 100 µL of stopping solution to each of the wells to stop color reaction.
15. Read the optical absorbance of the wells at 490 nm (or 492nm). The dose-response curve of this assay fits best to a 4 (or 5)-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4 (or 5)-parameter logistic function.
    Otherwise calculate mean absorbance values of wells containing standards and plot a standard curve on semi 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. 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 and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, reconstituted standard solution should be divided into test tubes in small amount and stored at or below -30ºC（stable for 1 month）.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2 -8°C.

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 vessel in assay and use a new tip for each sample to avoid cross contamination.

5. When sample concentration exceeds 100 ng/mL, it needs to be diluted with buffer solution to proper concentration.

6. During the incubation except color reaction, the test plate should be shaking gently by plate shaker to promote immunoreaction.

7. Perform all the determination in duplicate.

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

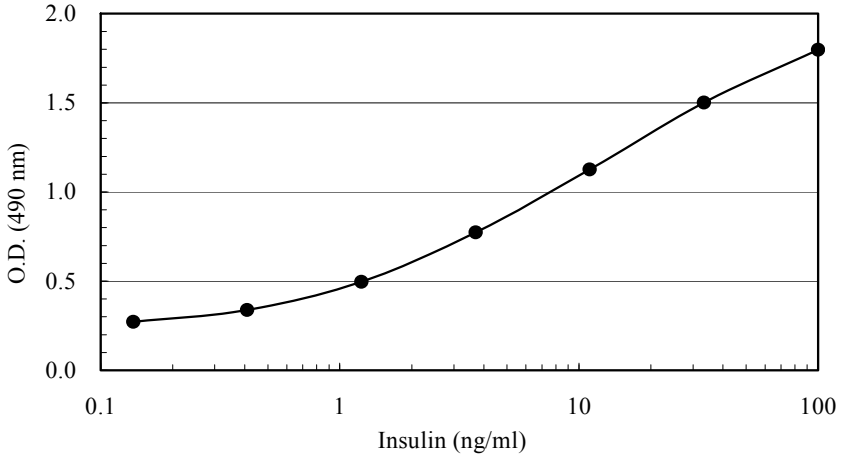
9. To quantitate accurately always run a standard curve when testing samples.

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.

Ⅵ. Performance Characteristics

Typical standard curve



<Analytical recovery>

<Human serum A>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	1.42		
0.37	1.83	1.79	102.23
3.33	4.77	4.75	100.42
10.00	11.55	11.42	101.14

<Human serum B>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	3.39		
0.37	3.77	3.76	100.27
3.33	5.85	6.72	87.05
10.00	10.65	13.39	79.54

<Human serum C>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	0.30		
0.37	0.68	0.67	101.49
3.33	3.52	3.63	96.97
10.00	10.07	10.30	97.77

<Human serum D>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	0.24		
0.37	0.53	0.61	86.89
3.33	3.13	3.57	87.68
10.00	7.86	10.24	76.76

<Human serum E>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	0.56		
0.37	0.88	0.93	94.62
3.33	3.55	3.89	91.26
10.00	8.81	10.56	83.43

<Human serum F>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	1.71		
0.37	2.11	2.08	101.44
3.33	4.83	5.04	95.83
10.00	11.04	11.71	94.28

<Rabbit serum A>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	0.20		
0.37	0.65	0.57	114.04
3.33	4.39	3.53	124.36
10.00	9.54	10.20	93.53

<Rabbit serum B>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	1.13		
0.37	1.70	1.50	113.33
3.33	4.16	4.46	93.27
10.00	11.95	11.13	107.37

<Rabbit serum C>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	0.58		
0.37	1.05	0.95	110.53
3.33	3.49	3.91	89.26
10.00	9.79	10.58	92.53

<Dog serum A>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	1.11		
0.37	1.70	1.48	114.86
3.33	7.09	4.44	159.68
10.00	17.11	11.11	154.01

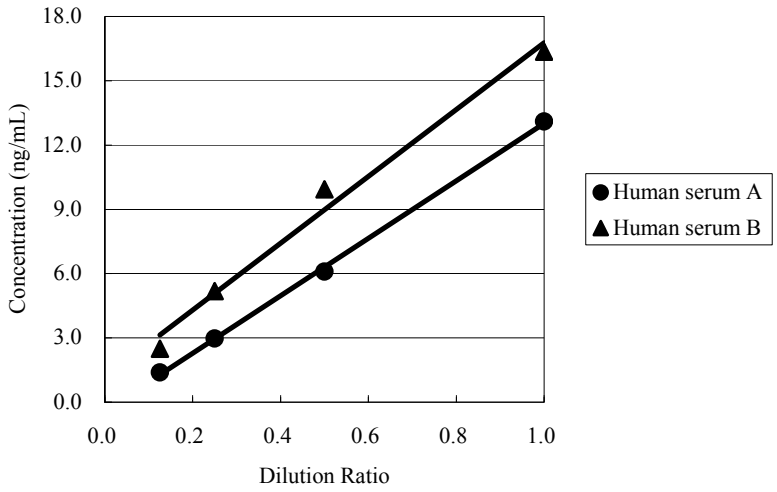
<Dog serum B>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	2.30		
0.37	2.81	2.67	105.24
3.33	7.31	5.63	129.84
10.00	16.20	12.30	131.71

<Dog serum C>

Added Insulin (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.00	1.76		
0.37	2.44	2.13	114.55
3.33	7.61	5.09	149.51
10.00	20.73	11.76	176.28

＜Dilution test＞



<Precision and reproducibility>

- Intra-assay CV(%)Human serum 6.59- 7.10, rabbit serum 2.51-9.08, dog serum 1.39-8.58
- Inter-assay CV(%)Human serum 6.86 -11.86

<Assay range>

0.137-100 ng/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 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. Reference

1. Bell GI, Pictet RL et al., Sequence of the human insulin gene. *Nature* ***284***: 26-32, 1980
2. Y. Zhang et al., Ionic mechanisms underlying abnormal QT prolongation and the associated arrhythmias in diabetic rabbits : A role of rapid delayed rectifier K^+ current. *Cell Physiol Biochem* **19**: 225-238, 2007

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Update at Apr. 13, 2012