DATA SHEET

YK060 Insulin ELISA

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 (IR-insulin) 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 HRP labeled streptoavidin (SA-HRP). 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 in the range of 0.137-100 ng/mL
- The assay completes 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 was 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
  12 months from the date of manufacturing.
  The expiry date is described on the label of kit.
  Store all of the components at 2-8℃.

Contents

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

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Ⅱ. Characteristics

This ELISA kit is used for quantitative determination of insulin in plasma 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 kit shows the following crossreactivities: 100% to human insulin, rabbit insulin and dog insulin, and 20% crossreactivity to human proinsulin.

< Test Principle >

This ELISA kit for determination of insulin (IR-insulin) in serum is based on a sandwich enzyme immunoassay. The 96-wells plate is coated with guinea pig anti human insulin antibody and insulin standard or samples are added to the wells for their immunoreaction. After incubation and plate washing, biotinylated guinea pig anti human insulin antibody is introduced to the wells and the antibody - antigen - labeled antibody complex is formed on the surface of the well. After rinsing out excessive labeled antibody, HRP labeled streptoavidin (SA-HRP) are added to bind 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	MTP*1	1 plate (96 wells)	Guinea pig anti human insulin antibody
2. Standard	lyophilized	1 vial (100ng)	Recombinant human insulin
3. Labeled antibody	liquid	1 bottle (12 mL)	Biotinylated guinea pig anti human insulin antibo
4. SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
5. Substrate buffer	liquid	1 bottle (24 mL)	0.015% hydrogen peroxide
6. OPD tablet	liquid	2 tablets	o -Phenylenediamine dihydrochloride
7. Stopping solution	tablet	1 bottle (12 mL)	1M H_2SO_4
8. Buffer solution	liquid	1 bottle (20 mL)	Phosphate buffer
9. Washing solution (Concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
10. Adhesive foil		3 sheets	

MTP*1..... Microtiter plate

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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 extinction 2.5 at 492 nm
3. Microtiter plate shaker
4. Test tubes for preparation of standard solution
5. Washing device for microtiter plate and dispenser with aspiration system
6. Graduated cylinder (1,000 mL)
7. Distilled water or deionized water

< Preparatory work >

1) Preparation of standard solution:
   Reconstitute the insulin standard (lyophilized, 100 ng/vial) with 1mL of buffer solution, which affords 100 ng/mL standard solution. Then, the 0.2ml of the standard solution is diluted with 0.4 mL of buffer solution that yields 33.33 ng/mL standard solution. Repeat the dilution to make each standard solution of 11.11, 3.70, 1.23, 0.41, 0.137 ng/mL. Buffer solution is used as 0 ng/mL.

2) Preparation of substrate solution:
   Resolve 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 1000 mL with distilled or deionized water.

4) Other reagents are ready for use.

< Procedure >

1. Bring all the reagents and samples return to room temperature before beginning the test.
2. Add 0.35mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further thrice (total four times).
3. Fill 150 µL of buffer solution into wells first and then introduce 25 µL of standard solutions (0, 0.137, 0.41, 1.23, 3.70, 11.11, 33.33 and 100 ng/mL) or samples into wells.
4. Cover the plate with adhesive foil and incubate it at 4℃ overnight for 16~20 hours. (Still, plate shaker not need)
5. After 4℃ incubation, move the plate to room temperature and waiting for half hour, take off the adhesive foil, aspirate and wash the wells four times with approximately 0.35 mL/well of washing solution.
6. Pipette 100 µL of labeled antibody into the wells.
7. Cover the plate with adhesive foil and incubate it for two hours at room temperature. During the incubation, the plate should be shake with a microtiter plate shaker.
8. Take off the adhesive foil, aspirate the solution in the wells and wash the wells four times with approximately 0.35 mL/well of washing solution.
9. Pipette 100 µL of SA-HRP solution into the wells.
10. Cover the plate with adhesive foil and incubate it at room temperature (20-30℃) for one hour. During the incubation, the plate should be shake with a plate shaker.
11. Resolve OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.
12. Take off the adhesive foil, aspirate the solution in the wells and wash the wells four times with approximately 0.35 mL/well of washing solution.
13. Pipette 100 µL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.
14. Add 100 µL of stopping solution into the wells to stop color reaction.
15. Read the optical absorbance of the wells at 492 nm. Calculate mean absorbance values of wells containing standards and plot a standard curve on logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance value). Use the standard curve to read insulin concentrations in samples from the corresponding absorbance values.

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. Insulin standard and substrate solution should be prepared immediately before use.

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, pipette standard solutions or samples into each well of plate precisely. Using clean test tubes or vessel in assay and use a new tip for each sample to avoid cross contamination.

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

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

7. During continuous shaking of test plate, the plate shaker may be heated up. It is recommended to place styrene foam or plywood between the plate and the shaker.

8. Perform all the determination in duplicate.

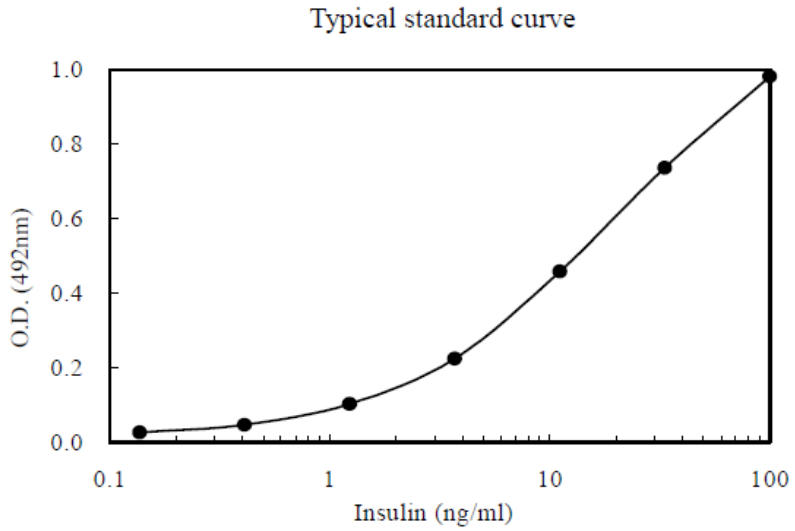
9. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

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 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

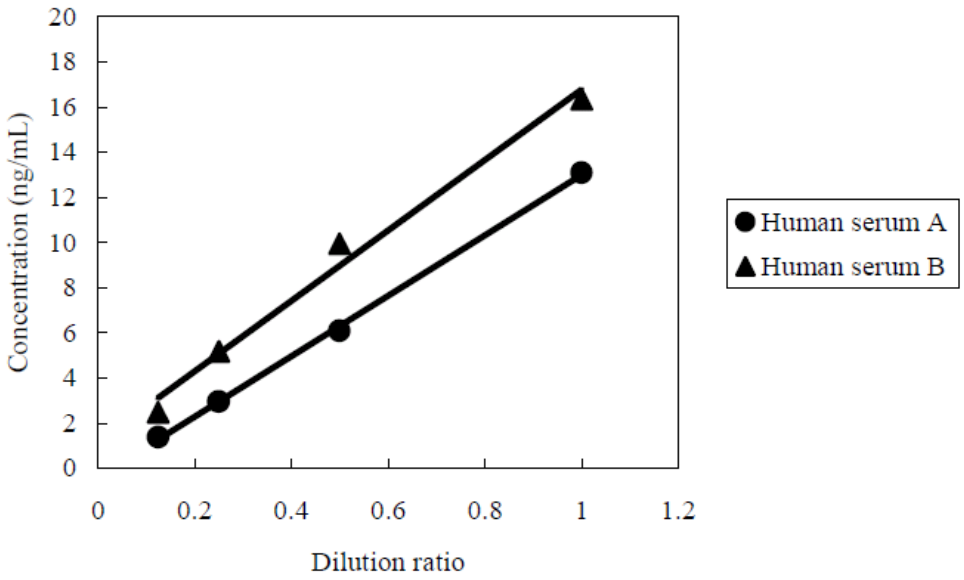
<Dog serum B>

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

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