

**KAMIYA BIOMEDICAL COMPANY**

# Dog, Human and Rabbit Insulin ELISA

**For the quantitative determination  
of insulin in dog, human and rabbit serum.**

**Cat. No. KT-438**

**For Research Use Only.**

**PRODUCT INFORMATION****Dog, Human and Rabbit Insulin ELISA**  
**Cat. No. KT-438****INTENDED USE**

The Dog, Human and Rabbit Insulin ELISA is for the quantitative determination of insulin in dog, human and rabbit serum. For research use only.

**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 the determination of insulin concentration (IR-insulin) in serum samples of human, rabbit and dog is based on a sandwich enzyme immunoassay by using a combination of guinea pig anti-human insulin antibody (coated on plate), recombinant insulin calibrator, 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. The kit is characterized by sensitive quantification, high specificity and no influences with other components in samples and needlessness of sample pretreatment. Human insulin calibrator is a recombinant product.

**PRINCIPLE**

This ELISA kit for determination of insulin (IR-insulin) in serum is based on a sandwich enzyme immunoassay. The 96-well plate is coated with guinea pig anti-human insulin antibody and insulin calibrator 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) is added to bind to labeled antibody. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of insulin is calculated.

**COMPONENTS**

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP <sup>*1</sup>	1 plate (96-well)	Guinea pig anti-human insulin antibody
2. Calibrator	Lyophilized	1 vial (100 ng)	Recombinant human insulin
3. Labeled Antibody	Liquid	1 bottle (12 mL)	Biotinylated guinea pig anti-human insulin Ab
4. Substrate Buffer	Liquid	1 bottle (24 mL)	0.015% Hydrogen peroxide
5. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
6. Stop Solution	Liquid	1 bottle (12 mL)	1M H <sub>2</sub> SO <sub>4</sub>
7. Buffer Solution	Liquid	1 bottle (20 mL)	Phosphate buffer
8. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin
9. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
10. Plate Seal		3 sheets	

MTP<sup>\*1</sup>..... Microtiter plate

## MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 492 nm
- Rotator for microtiter plate
- Washing device for microtiter plate and dispenser with aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Test tubes for preparation of Calibrator Solution
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

## PRECAUTIONS

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

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample and calibrator to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

## REAGENT PREPARATION

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

Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.

2. Preparation of Substrate Solution: Dissolve one OPD Tablet in 11 mL of Substrate Buffer.

Note: Substrate Solution must be prepared immediately before assay. Use clean test tubes or vessels.

3. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water. Diluted Wash Solution is stable for 6 months at 4°C.

Note: During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted. Diluted washing solution is stable for 6 months at 4°C.

4. Other reagents are ready for use.

## STORAGE

Store kit at 4°C.

## SPECIMEN COLLECTION AND HANDLING

Samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amounts and frozen at or below -30°C. Avoid repeated freeze/thaw cycles.

## ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Add 0.35 mL/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 calibrator 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 a Plate Seal and incubate it at 4°C overnight for 16~20 hours. (Still, plate shaker not needed)

5. After 4°C incubation, move the plate to room temperature and wait for half hour, take off the Plate Seal, 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 a Plate Seal and incubate it for two hours at room temperature. During the incubation, the plate should be shaken with a microtiter plate shaker. 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. During incubation except color reaction, the test plate should be shaking gently by plate shaker to promote immunoreaction.
8. Take off the Plate Seal, 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 a Plate Seal and incubate it at room temperature (20-30°C) for one hour. During the incubation, the plate should be shaken 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 Plate Seal, 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 a Plate Seal and incubate it for 30 minutes at room temperature.
14. Add 100 µL of stop solution into the wells to stop color reaction.
15. Read the optical absorbance of the wells at 492 nm. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

Note: Perform all determinations in duplicate.

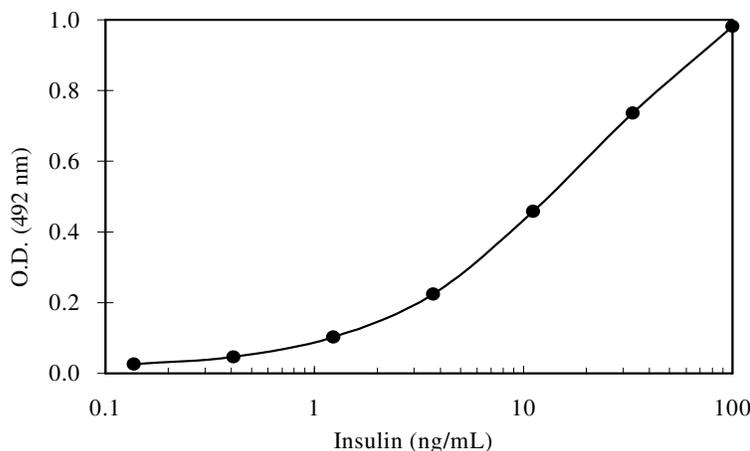
## RESULTS

Calculate mean absorbance values of wells containing the Calibrators and plot a calibration curve on logarithmic graph paper (abscissa: concentration of Calibrators; ordinate: absorbance values of Calibrators). Use the calibration curve to read insulin concentrations in samples from the corresponding absorbance values.

When a sample value exceeds 100 ng/mL, it needs to be diluted with buffer solution until the value is within the assay range.

## PERFORMANCE

**Typical Calibration Curve** (example only, a new calibration curve for each run must be established by the end-user)

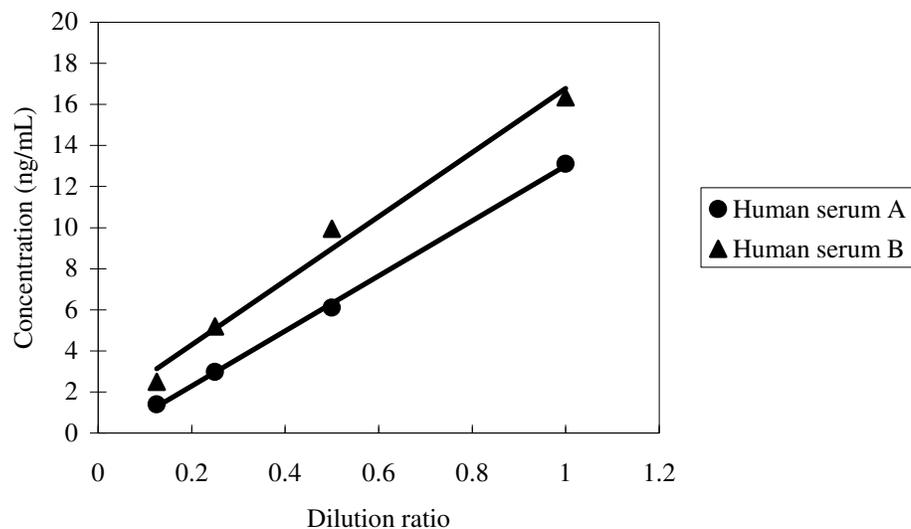


**Analytical Recovery**

Sample	Insulin Added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Human serum A	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	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	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	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	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	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	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	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	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	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	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	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

**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

**Dilution Test****Assay Range**

0.137- 100 ng/mL

**Cross-Reactivity**

This kit shows the following cross-reactivities: 100% to human insulin, rabbit insulin and dog insulin, and 20% cross-reactivity to human proinsulin.

**FOR RESEARCH USE ONLY****KAMIYA BIOMEDICAL COMPANY**

12779 Gateway Drive, Seattle, WA 98168

Tel: (206) 575-8068 Fax: (206) 575-8094

Email: LifeScience@k-assay.com

www.k-assay.com