



# Insulin (Human) ELISA Kit

Catalog Number KA0921

96 assays

Version: 04

Intended for research use only

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## **Introduction**

### **Intended Use**

The Insulin (Human) ELISA Kit is intended for the quantitative measurement Insulin in human serum or plasma.

### **Background**

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the  $\beta$ -cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain. Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

### **Principle of the Assay**

The Insulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation insulin in the sample reacts with enzyme (HRP)-conjugated anti-insulin antibody and anti-insulin antibody bound to micro-titration well. A simple washing step removes unbound enzyme labeled antibody. The bound HRP complex is detected by reaction with TMB substrate. The reaction is stopped by adding acid to give a colorimetric endpoint that is read using ELISA reader.

## General Information

### Materials Supplied

List of component

Component	Amount
Microwells coated with Insulin MAb	96 (8x12) wells
Insulin Standard 1: 1 vial (Ready to use)	2 ml
Insulin Standards 2: 1 vial (Ready to use)	1 ml
Insulin Standards 3-6: 4 vials (Ready to use)	0.5 ml
Insulin Enzyme Conjugate: 1 vial	1 ml
Assay Diluent: 1 bottle (Ready to use)	14 ml
TMB Substrate: 1 bottle (Ready to use)	12 ml
Stop Solution: 1 bottle (Ready to use)	12 ml
20X Wash concentrate: 1 bottle	25 ml

### Storage Instruction

- ✓ Store the kit at 2-8°C.
- ✓ Keep microwells sealed in a dry bag with desiccants.
- ✓ The reagents are stable until expiration of the kit.
- ✓ Do not expose reagent to heat, sun, or strong light.

### Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

### Precautions for Use

- Precautions
- ✓ Potential biohazardous materials:

The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other

infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.

- ✓ Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
  - ✓ The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
  - ✓ It is recommended that standards, control and serum samples be run in duplicate.
  - ✓ Optimal results will be obtained by strict adherence to the test protocol. Accurate and precise pipetting as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
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- Limitation
  - ✓ Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

## **Assay Protocol**

### **Reagent Preparation**

- ✓ 20X Enzyme Conjugate: Prepare 1X working dilution at 1:20 with assay diluent as needed, e.g. 0.1 ml of the stock conjugate in 1.9 ml of assay diluent is sufficient for 20 wells. The diluted conjugate has to be used the same day.
- ✓ 20X Wash Buffer Concentrate: Prepare 1X wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1X wash buffer at room temperature.

### **Sample Preparation**

- ✓ Collect blood specimens and separate the serum immediately.
- ✓ Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
- ✓ Avoid multiple freeze-thaw cycles.
- ✓ Prior to assay, frozen sera should be completely thawed and mixed well.
- ✓ Do not use grossly lipemic specimens.

### **Assay Procedure**

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 µl of Insulin standards, control and patient's sera into appropriate wells.
3. Add 100 µl of working Insulin Enzyme Conjugate to all wells.
4. Thoroughly mix for 10 sec., it is important to have a complete mixing in this step.
5. Incubate for 60 minutes at room temperature (18-26°C).
6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

## Data Analysis

### Calculation of Results

The standard curve is constructed as follows:

1. Check Insulin standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit.
2. To construct the standard curve, plot the absorbance for the insulin standards (vertical axis) versus the insulin standard concentrations in  $\mu\text{IU/ml}$  (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Value above the highest point of the standard are retested after diluting with "0" standard.

- Example of Standard Data

	OD 450 nm	Conc. $\mu\text{IU/mL}$
Std 1	0.05	0
Std 2	0.11	6.25
Std 3	0.22	12.5
Std 4	0.49	25
Std 5	1.00	50
Std 6	2.11	100

- Expected Values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the Insulin (Human) ELISA Kit the following values are observed:  $< 25 \mu\text{IU/ml}$ .

### Performance Characteristics

- Correlation with a Reference ELISA kit:

A total of 62 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

Correlation	Slope	Intercept
0.91	0.80	0.24

- Precision

- Intra-Assay

Sample	No. of Replicates	Mean $\mu$ IU/ml	Standard Deviation	Coefficient of Variation (%)
1	10	9.26	0.58	6.3
2	10	7.01	0.57	8.1

- Inter-Assay

Sample	No. of Replicates	Mean $\mu$ IU/ml	Standard Deviation	Coefficient of Variation (%)
1	10	6.79	0.58	8.5
2	10	9.27	0.69	7.4

- Linearity

Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4, 1:8. Insulin values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

Original Value		Percentage of Recovery		
Serum	( $\mu$ IU/ml)	1/2	1/4	1/8
1	30	95.1	91.5	88.6
2	71	106	95.5	105

- Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean ( $\mu$ IU/ml)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	20	0.477	0.495	1.467

- Specificity

The antibodies employed in this kit cross react with bovine insulin (20-25%) and porcine insulin but not with proinsulin of any species or any other insulin complexes.

- Recovery

Samples have been spiked by adding Insulin solutions with known concentrations in a 1:1 ratio.

Expected value ( $\mu$ IU/ml)	Recovered ( $\mu$ IU/ml)	Percentage of Recovery
9.85	8.80	89.3
41.1	40.4	98.3
53.7	54.2	100.9

## Resources

### References

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**Plate Layout**

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