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Product Information

Insulin ELISA

Catalog Number **SE120069**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the β-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 contains 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 disulfide bridges. There is also an intra-chain disulfide 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.

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

The Insulin ELISA Kitis 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 the microwell. 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.

Components

Materials Provided	96 Tests
Microwell coated with Insulin MAb	12 × 8 × 1
Insulin Standard 1: 1 vial (ready to use)	2 mL
Insulin Standard 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
20× Wash concentrate: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

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

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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

20× Wash Buffer Concentrate
Prepare 1× wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1× wash buffer at room temperature.

20× Enzyme Conjugate
Prepare 1× working solution by diluting 20-fold with assay diluent as needed (*e.g.*, 0.1 mL of the 20× Enzyme Conjugate in 1.9 mL of Assay Diluent is sufficient for 20 wells). The diluted conjugate has to be used the same day.

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: 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.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18–26 °C).

1. Place the desired number of coated strips into the holder.
2. Pipette 25 µL of Insulin standards, control, and sera into appropriate wells.
3. Add 100 µL of 1× working Insulin Enzyme Conjugate to all wells.
4. Thoroughly mix for 10 seconds, 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 1× 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.

Results

Calculations

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 the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the insulin standards (vertical axis) versus the insulin standard concentrations in μIU/mL (horizontal axis) on a linear graph paper. Draw the best curve through the points.

Example of Standard Data

	OD 450 nm	**Concentration μIU/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

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.

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 ELISA the following values are observed: <25 μIU/mL.

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient’s history, physical findings, and other diagnostic procedures.

Product Profile

Correlation

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

Serum	No. of Replicates	Mean μIU/mL	Standard Deviation	Coefficient of Variation (%)
1	10	9.26	0.58	6.3
2	10	7.01	0.57	8.1

Inter-Assay

Serum	No. of Replicates	Mean μIU/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	(μIU/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 2 SD of the standard zero point tested 20 times in the same run.

Specificity

The antibodies employed in this kit crossreact 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 (μIU/mL)	Recovered (μIU/mL)	Percentage of Recovery
9.85	8.80	89.3
41.1	40.4	98.3
53.7	54.2	100.9

References

1. Ashby, J., and Frier, B., Circulating C-Peptide: Measurement and Clinical Applications. Annals of Clinical Biochemistry, 18:125, 1981.
2. Beischer, W., Proinsulin and C-Peptide in Humans. In Hormones in Normal and Abnormal Human Tissues. Volume 3K, Fotherby and Pal, S., eds. Walter DeGruyter, (Berlin, Germany: 1983). pp. 1-43.
3. Beyer, J. et al., C-Peptide: Its Biogenesis, Structure, Determination and Clinical Significance. Giornale Italiano di Chimica Clinica, 4 Supp. 9:22, 1979.
4. Bonger, A., and Garcia-Webb, P., C-Peptide Measurement: Methods and Clinical Utility. CRC Critical Reviews in Clinical Laboratory Sciences, 19:297, 1984.
5. Chevenne D. et.al., Immunoradiometric Assay of Human Intact Proinsulin Applied to Patients with Type 2 Diabetes, Impaired Glucose Tolerance, and Hyperandrogenism. Clinical Chemistry, 40/5:754, 1994
6. Bowsher, R.R. et al., A Rapid and Sensitive Radioimmunoassay for the Measurement of Proinsulin in Human Serum. Diabetes, 41:1084, 1992.
7. Kao, P.C. et al., Proinsulin by Immunochemi-luminometric Assay for the Diagnosis of Insulinoma. Jorunal of Clinical Endocrinology and Metabolism, 78:1048, 1994.
8. Dhahir, F.J. et al., Amplified Enzyme-Linked Immunoassay of Human Proinsulin in Serum (Detection Limit: 0,1 pmol/L). Clinical Chemistry, 38/2:227, 1992.

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