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Product Information

Insulin ELISA kit

Catalog Number: EA100870
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Insulin 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 ß-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 Test

The OriGene Insulin ELISA is based on solid phase sandwich ELISA method. The samples and conjugate reagent (anti-Insulin biotin & HRP) are added to the wells coated with Streptavidin. Insulin in the serum binds to the matched pair Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and HRP conjugate are washed off through a washing step.
Upon addition of the substrate, the intensity of color is proportional to the concentration of Insulin in the samples. A standard curve is prepared by relating the color intensity to the concentration of Insulin.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell coated with Streptavidin	12x8x1
2. Insulin Standards: 6 vials (ready to use)	0.5ml
3. Insulin Conjugate Reagent: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. 20X Wash concentrate: 1 bottle	25ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Storage and Stability

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

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. 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.
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.

Reagent Preparation

1. **Prepare 1X Wash buffer**: Add the Wash Buffer (20X, 25 ml) to 475 ml of distilled or de-ionized water. Store at room temperature (18-26°C).

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 25µl of Insulin standards, control and sera into appropriate wells.
3. Add 100µl of Insulin Conjugate Reagent to all wells. Mix well, for 20 seconds.
4. Incubate for 60 minutes at room temperature (20-25°C).
5. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100µl of TMB substrate into all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

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 µIU/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 Curve

1. The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Conc. µIU/mL	OD450
Std 1	0	0.007
Std 2	5	0.113
Std 3	25	0.526
Std 4	50	0.914
Std 5	100	1.397
Std 6	300	2.225

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.

Limitations of the Test

1. 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.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

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