ORIGENE

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Product Information

Pro-Insulin ELISA kit

Catalog Number: EA100873
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Pro-Insulin ELISA Kit is intended for the quantitative measurement of insulinomas in human serum or plasma.

Background

Proinsulin is a 9390 MW polypeptide of 86 amino acids that is synthesized in the ß cells of the pancreas and is the precursor molecule for insulin. Most proinsulin is converted to insulin and C-Peptide, which are secreted in equimolar amounts into the blood. About 15 % is not converted and is released as proinsulin. The biological activity of proinsulin is only about 10% of Insulin, but the half life of proinsulin is three times as long as insulin. The level of proinsulin in serum can be a reflection of ß cell status. Both IDDM and NIDDM are characterized by dysfunction of the pancreatic ß cells. Elevated proinsulin levels have been noted at the onset of IDDM and in healthy siblings of IDDM patients. Proinsulin levels may also be increased in patients with established NIDDM. Increased levels of circulating proinsulin are found in older patients, pregnant or obese diabetics, and patients with insulinomas, functional hypoglycemia and hyperinsulinemia, a rare syndrome. Because the structure of proinsulin is similar to insulin, proinsulin may be detected as immunoreactive insulin in the insulin assay. Immunoreactive insulin levels are generally determined in conventional RIA's, which overestimate the insulin level because the methods use antibodies which cross-react with proinsulin. By calculating the molar ration of proinsulin to true insulin (P/I), a better assessment of ß cell function can be made.

Principle of the Test

The Proinsulin EIA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Proinsulin molecule. An aliquot of patient sample containing endogenous Proinsulin is incubated in the coated wells. After washing off the samples in a second step an enzyme conjugate, which is an anti-Proinsulin antibody conjugated with horseradish peroxidase is incubated in the wells. After incubation the unbound conjugate is washed off with wash solution. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Proinsulin in the patient sample.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with anti Pro-insulin Antibody	12x8x1
2. Pro-Insulin Standards: 6 vials (ready to use)	1 ml

3. Pro-Insulin Enzyme Conjugate 11X: 1 vial	1.2 ml
4. TMB Substrate: 1 bottle (ready to use)	14 mL
5. Stop Solution: 1 bottle (ready to use)	14 ml
6. Wash concentrate 40X: 1 bottle	30 ml
7. Sample Diluent : 1 vial (ready to use)	2 mL
8. Conjugate Diluent: 1 bottle (ready to use)	12 mL
9. control (low & high)	2 ml
10. Assay Buffer: 1 bottle (ready to use)	12 mL

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

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. Plasma should be treated with EDTA.
3. Plasma samples may be stored at 2-8°C for up to 8 hours, and should be frozen at -20°C or lower for up to 4 months.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Do not use grossly hemolyzed or lipemic specimens.

Reagent Preparation

1. **Prepare 1X Wash buffer:** Add the Wash Concentrate (30 mL,40X) to 1170 mL of distilled or deionized water. Store at room temperature.
2. **Prepare 1X Enzyme Conjugate:** Dilute the concentrated Enzyme Conjugate (11X) in the Conjugate Diluent, e.g 100 µl Enzyme Conjugate + 1000 µl Conjugate Diluent. 100 µl diluted Enzyme Conjugate is added into each well. The diluted Enzyme Conjugate is stable for 24 h at room temperature.

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- 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

1. Secure the desired number of coated Microtiter wells in the holder.

2. Dispense 100 µl of Proinsulin standards, control and samples into appropriate wells.
3. Dispense 100 µl of Assay buffer into each well.
4. Mix thoroughly for 10 seconds. It is important to achieve a complete mixing in this step.
5. Cover the plate with a plate sealer and incubate overnight (16-24 hours) at 4°C in a humidity chamber.
6. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (350 µl per well). Strike the Wells sharply on absorbance paper to remove residual droplets.
7. Dispense 100 µl of diluted Enzyme-Conjugate into each well.
8. Mix thoroughly for 10 seconds. It is important to achieve a complete mixing in this step.
9. Incubate for 60 minutes at room temperature without agitation.
10. Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (350 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
11. Add 100 µl of Substrate Solution to each well at timed intervals.
12. Incubate for 30 minutes at room temperature.
13. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well
14. Read the OD at 450 nm within 15 minutes after adding the stop solution.

Calculation of Results

The standard curve is constructed as follows:

1. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in pmol/l with absorbance value on the Y-axis and concentration on the X-axis.
2. Calculate the average absorbance values for each set of reference standards, controls and patient samples.
3. Using the mean absorbance value for each sample determine the corresponding concentration of Proinsulin in pmol/l from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further converted by the appropriate dilution factor.
5. If in an initial assay, a specimen is found to contain more proinsulin than the upper limit of the standard curve, the specimens must be diluted with Sample diluent.

Expected Values

1. Normal range for serum and plasma

It is recommended that each laboratory establishes its own range of normal Proinsulin levels. The normal range values observed with DRG Proinsulin ELISA KIT with normal adult males and females are as follows:

	N	Age ± SD	Mean ± SD
Post 12-hour Fasting (Plasma)	32	-	4,5 ± 3,8
Post 12-hour Fasting (Serum)	15	32 ± 11	2,5 ± 1,8

Additionally, a glucose tolerance test was performed post 12-hour fasting with 77 healthy children (Age 14 ± 3). Serum was drawn after 12 hours of fasting. Participants were then administered 75 grams of glucose and samples again drawn after 30-120 minutes.

	Mean (± 1SD)*pmol/L
Post 12 hour Fasting (Serum)	1,3 (0,5 - 3,5)
30 min. after Glucose administration	6,4 (3,0 - 13,6)
120 min. after Glucose administration	14,8 (6,5 - 33.3)

* for logarithmic normal distribution

2. Example of a typical standard curve
The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standards	Conc (pmol/L)	Optical Units
Standard 0	0	0.16
Standard 1	2.6	0.25
Standard 2	6.6	0.36
Standard 3	16.5	0.63
Standard 4	33	1.06
Standard 5	66	1.82

Performance Characteristics

1. Sensitivity
The minimal detectable concentration of human proinsulin by this assay is estimated to be 0,5 pmol/l - overnight incubation; 1 pmol - incubation 3h at RT.

References

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