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ProInsulin (human) ELISA Kit

(Catalog # K7433-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

Proinsulin is synthesized in the beta 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 beta 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, 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 beta cell function can be made. BioVision's Proinsulin kit 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 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 color developed is proportional to the concentration of Proinsulin in the sample.

II. Application:

Quantitative measurement of insulinomas in human serum or plasma.

III. Specificity:

Human ProInsulin

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K7433-100	Part No.
Microwell coated with anti Proinsulin Ab	12 stripsx8x1	K7433-100-1
Proinsulin Standard*	6x1 ml	K7433-100-2
Enzyme Conjugate (11X)	1.2 ml	K7433-100-3
Wash Concentrate (40X)	30 ml	K7433-100-4
Sample Diluent	2 ml	K7433-100-5
Conjugate Diluent	12 ml	K7433-100-6
Control (low & high)	2 ml	K7433-100-7
Assay Buffer	12 ml	K7433-100-8
TMB Substrate	14 ml	K7433-100-9
Stop Solution	14 ml	K7433-100-10

* Check Proinsulin standard value on each standard vial. This value might vary from lot to lot.

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{o}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Concentrate:** Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).
- **Enzyme Conjugate:** Dilute the concentrated Enzyme Conjugate in the Conjugate Diluent.100 µl Enzyme Conjugate + 1000 µl Conjugate Diluent). For each well you need 100 µl diluted Enzyme Conjugate. The diluted Enzyme Conjugate is stable for 24 hr at room temperature.

VIII. Warning & Precautions:

- Potential biohazardous materials: The calibrator & controls contains 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.
- 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 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.

IX. Sample Preparation and Storage:

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.

X. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 100 µl of Proinsulin Standard, control, and samples into designated wells.
3. Dispense 100 µl of Assay buffer into each well.
4. Mix thoroughly for 10 sec. It is important to achieve a complete mixing in this step.
5. Cover the plate with a plate sealer and incubate overnight (16-24 hrs) 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 sec. It is important to achieve a complete mixing in this step.
9. Incubate for 60 min. 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 min. at room temperature.
13. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well
14. Read the OD at 450±10 nm within 15 min. after adding the stop solution.

XI. Calculation: Construct the standard curve; plot the absorbance for the Proinsulin standards (vertical axis) versus Proinsulin standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. Any diluted samples must be further converted by the appropriate dilution factor. 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.

Example of a Standard Curve:

Standard	OD
Standard 1 (0 ng/ml)	0.16
Standard 2 (2.6 ng/ml)	0.25
Standard 3 (6.6 ng/ml)	0.36
Standard 4 (16.5 ng/ml)	0.63
Standard 5 (33 ng/ml)	1.06
Standard 6 (66 ng/ml)	1.82

Expected Values: It is recommended that each laboratory establish its own range of Proinsulin levels. The following values for Proinsulin were established by the BioVision with normal adult males & females

	N	Age ± SD	Mean ± SD pmol/l
Post 12 hr fasting (plasma)	32	-	4.5±3.8
Post 12 hr 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 min.

	Mean (± 1SD)* pmol/l
Post 12 hr fasting (plasma)	1.3 (0.5-3.5)
30 min. after glucose administration	6.4 (3-13.6)
120 min. after glucose administration	14.8 (6.5-33.3)

* -for logarithmic normal distribution

XII. RELATED PRODUCTS:

Leptin (human) ELISA Kit (4777)
Insulin (human) ELISA Kit (K4742)
Irisin Competitive ELISA Kit (4761)
C-Peptide (human/mouse/rat) EIA Kit (K4757)

FOR RESEARCH USE ONLY! Not to be used on humans.