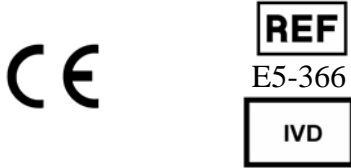




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### Pro-Insulin ELISA



FOR INVITRO DIAGNOSTIC USE ONLY

#### INTENDED USE

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

#### SUMMARY AND EXPLANATION

The Immunospec Proinsulin is a 9390 MW polypeptide of 86 amino acids, that 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  $\beta$  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 RIAs, which overestimate the insulin level because the methods use antibodies which crossreact with proinsulin. By calculating the molar ration of proinsulin to true insulin (P/I), a better assessment of  $\beta$  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.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated with anti Pro-insulin Antibody	12x8x1
2.	Pro-Insulin Standards: 6 vials (ready to use)	1ml
3.	Pro-Insulin Enzyme Conjugate 11X: 1 vial	1.2 ml
4.	TMB Substrate: 1 bottle (ready to use)	14mL
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 NOT PROVIDED

1. Distilled or deionized water
2. precision pipettes, Disposable pipette tips
3. Micortiter well reader capable of reading absorbance at 450nm
4. Absorbance paper or paper towel
5. 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 test reagents to heat, sun, or strong light.

#### WARNINGS AND PRECAUTIONS

1. 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.
2. This test kit is USA FDA exempt product.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. 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.

#### 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.

#### REAGENTS PREPARATION

1. Prepare 1X Wash buffer by adding the contents of the bottle (25 mL, 20X) to 475 mL of distilled or deionized water. Store at RT.
2. Dilute the concentrated Enzyme Conjugate in the Conjugate Diluent.(100  $\mu$ l Enzyme Conjugate + 1000  $\mu$ l Conjugate Diluent) For every well you need 100  $\mu$ l diluted Enzyme Conjugate. The diluted Enzyme Conjugate is stable for 24 h at room temperature.

#### ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Secure the desired number of coated *Microtiterwells* in the holder.
2. Dispense 100  $\mu$ l of Proinsulin *Standards*, control and samples into appropriate wells.
3. Dispense 100  $\mu$ l 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  $\mu$ l per well). Strike the Wells sharply on absorbance paper to remove residual droplets.
7. Dispense 100  $\mu$ l 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  $\mu$ l per well). Strike the wells sharply on absorbent paper to remove residual droplets.
11. Add 100  $\mu$ l 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  $\mu$ l of *Stop Solution* to each well
14. Read the OD at 450±10 nm within 15 minutes after adding the stop solution.

#### CALCULATION OF RESULTS

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 vertical(Y) axis and concentration on the horizontal (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. 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 Immunospec Proinsulin ELISA KIT with normal adult males and females are as follows:

	N	Age ± SD	Mean ± SD pmol/l
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.

Standard	Optical Units
Standard 0 (0 pmol/l)	0.16
Standard 1 (2.6 pmol/l)	0.25
Standard 2 (6.6 pmol/l)	0.36
Standard 3(16.5 pmol/l)	0.63
Standard 4 (33 pmol/l)	1.06
Standard 5 (66 pmol/l)	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.

##### 2. Specificity

The following peptides were tested for cross-reactivity of the assay:

Peptide	Produced Color Intensity Equivalent to Proinsulin in Serum (pmol/l)
Proinsulin 32 - 33 split, 500 pmol/l	7.5
Proinsulin 32 - 33 split, 5 pmol/l	0.
Proinsulin Des 31 - 32, 500 pmol/l	4.5
Proinsulin Des 31 - 32, 5 pmol/l	0
Proinsulin 65 - 66 split, 500 pmol/l	275
Proinsulin 65 - 66 split, 5 pmol/l	2.7
Proinsulin Des 64 - 65, 500 pmol/l	266
Proinsulin Des 64 - 65, 5 pmol/l	3.2
Proinsulin 56 - 57 split, 500 pmol/l	375
Proinsulin 56 - 57 split, 5 pmol/l	3.5
Proinsulin Des 57 - 65, 500 pmol/l	271
Proinsulin Des 57 - 65, 5 pmol/l	3.4
Human Insulin, 17000 pmol/l	0
Porcine Proinsulin 2,5 µg/ml	0
Bovine Proinsulin 2,0 µg/ml	0
Rat Proinsulin of Insulin, 160 pmol/l	0
Human C-Peptide, 33000 pmol/l	0
Proinsulin of Somatomedin-C, 10 µg/ml	0
Somatomedin C, 1 µg/ml	0

3. Precision: Intra-assay  
Inter-assay

Serum	n	Mean pmol/l	CV %	n	Mean pmol/l	CV%
1	10	6.97	4.3	10	7.32	6.8
2	10	27.2	2.9	10	29.6	5.5
3	10	60.3	7.4	10	64.7	5.5

4. Accuracy: The accuracy of the assay was evaluated by recovery and dilution tests.

##### Recovery

Serum	Endogenous Proinsulin pmol/l	Added Proinsulin pmol/l	Recovery %
1	6,8	10, 30, 50	101, 100, 93
2	27,2	10, 30, 50	93, 96, 95

##### 5. Recovery of Proinsulin in samples with different content of Insulin

To various serum samples with known proinsulin levels different quantities of insulin were given.

Insulin added pmol/l	Expected Proinsulin conc. pmol/l	Recovery %	Expected Proinsulin conc. pmol/l	Recovery %	Expected Proinsulin conc. pmol/l	Recovery %
0	51,6	100	15,1	100	5,0	100
72	51,6	98	15,1	98	5,0	106
215	51,6	100	15,1	97	5,0	106
636	51,6	96	15,1	97	5,0	100
1892	51,6	93	15,1	87	5,0	98
5676	51,6	73	15,1	76	5,0	81
17200	51,6	37	15,1	44	5,0	52

##### Recovery of Proinsulin in samples with different content of C-Peptide

To various serum samples with known proinsulin levels different quantities of C-peptide were given.

C-Peptide added pmol/l	Expected Proinsulin conc. pmol/l	Recovery %	Expected Proinsulin conc. pmol/l	Recovery %
0	51,6	100	5,3	100
16,6	51,6	99	5,3	102
166	51,6	101	5,3	96
1660	51,6	95	5,3	100

##### b. Linearity

Two patient samples were serially diluted with sample diluent in a linearity study. The average recovery was 102.9%.

Patient Number	Dilution	Expected Concentration pmol/l	Observed Concentration pmol/l	Recovery %
1	Undiluted	72.65	72.65	100.0
	1:2	36.33	36.88	101.5
	1:4	18.17	18.72	103.0
	1:8	9.09	9.33	102.7
	1:16	4.55	4.63	101.9
2	Undiluted	61.86	61.86	100.0
	1:2	30.93	31.01	100.3
	1:4	15.47	16.02	103.6
	1:8	7.74	8.06	104.1
	1:16	3.87	4.12	106.5

#### QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

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