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high sensitivity diagnostics

IV2-102E

English

Invitron Intact Proinsulin ELISA Kit

For in-vitro diagnostic use only

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IFU-2-102E_7.0

Definitions

	Consult instructions for use
REF	Catalogue number
	Use by date
LOT	Lot/Batch Code
	Storage temperature limitations
IVD	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <N> tests

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Invitron Intact Proinsulin ELISA Kit

Intended Use

The Invitron Intact Proinsulin ELISA kit is an immunometric assay for the quantitative measurement of intact proinsulin in human samples. Measurements of proinsulin are used in the diagnosis and treatment of patients with type 2 diabetes.

Summary and Explanation

Proinsulin is a precursor molecule for insulin and is synthesized by the pancreatic β-cells. Under normal circumstances, virtually all proinsulin is cleaved at residues 32-33 and 65-66 to produce insulin during the formation of secretory granules. Some unmodified proinsulin is released into the circulation, though it is believed to have little or no biological activity. Increased concentrations of circulating proinsulin may occur in insulin-resistant syndromes such as type 2 diabetes and in patients with insulinoma. When used in conjunction with a highly specific insulin assay, it may provide useful information on changes in the processing of insulin in such situations.

Principle

The Invitron Intact Proinsulin Assay is a two-site enzyme-linked immunosorbent assay (ELISA), employing a specific solid phase antibody immobilised on microplate wells and a soluble antibody labelled with biotin. The sample is incubated in the microplate well together with a buffer and, after a wash step, the biotin labelled antibody solution is added. After a second incubation and wash step, HRP labelled streptavidin is added. A third incubation and wash is followed by the addition of substrate solution. Following colour development “stop reagent” is added and the colour intensity measured in a 96-well microplate reader.

Materials Provided

- Coated Microplate Plate (12 x 8 wells).
  Microplate wells coated with a specific monoclonal antibody.
- Biotin Conjugated Antibody (12mL).
  Biotin labelled antibody in phosphate buffer. Supplied ready to use.
- HRP Labelled Streptavidin (12mL).
  HRP enzyme labelled streptavidin. Supplied ready to use.
- Standards (5 x 1ml lyophilized).
  5 concentrations – (typically) 0.00, 1.2, 5.5, 30.0, 110 pmol/l – Recombinant intact proinsulin in a buffer matrix, lyophilized and sealed under vacuum for stability. Refer to the Certificate of Analysis provided with the kit for exact concentrations. ***The standards are calibrated against WHO 1st International Standard for Proinsulin (IRR 09/296).***

- Controls (2 x 1ml lyophilized).
  Samples containing low (A) and high (B) concentrations of recombinant human proinsulin in a buffer matrix. Target values are provided in the Certificate of Analysis provided with the kit. *Each laboratory should establish its own expected concentration range.*
- Sample Buffer (12mL).
  Ready to use. Protein matrix including preservatives and 0.05% sodium azide.
- Substrate Solution (12mL).
  Tetramethylbenzidine (TMB) substrate. Supplied ready to use.
- Stop Reagent (12mL).
  ELISA stop reagent. Supplied ready to use.
- Wash Buffer Concentrate (50mL).
  Phosphate buffer including detergents. 30x concentrate.
- Product Insert

Material required but not supplied

- Deionized water
- Precision pipettes and disposable tips to deliver 10-1000 µL
- Microplate sealers
- A multi-channel dispenser or repeating dispenser
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microplate reader at 450nm, reference at 620/650nm

Warnings and Precautions

- For *in-vitro* diagnostic use only. For professional use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves and appropriate protective clothing when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Once components have been opened or reconstituted, they can be used within a four-week period, provided they have been stored at 2-8ºC.
- Do not mix or use components from kits with different lot numbers.
- This kit contains no human-derived material.

Preparation, Storage & Stability of Reagents

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. Microplate wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above. Reconstituted/diluted reagents are stable for 4 weeks when stored at 2-8°C.

Standards and Controls

Reconstitute each of the standards and controls by the addition of 1.0ml of deionized water. Allow these to stand for 5 minutes, then mix gently to ensure all solids are dissolved. Stability of the reconstituted Standards is four (4) weeks when stored at 2-8°C.

Wash Buffer

Make up working strength Wash Buffer by diluting 1 part of Wash Buffer concentrate with 29 parts of deionized water.

Specimen Collection & Storage

Invitron recommend using heparin or EDTA Plasma for intact proinsulin measurements. Full recovery of intact proinsulin cannot be achieved from serum samples. Do not use severely haemolysed specimens.

Specimen Collection

Plasma: Whole blood should be collected into a tube containing EDTA or heparin anticoagulant and centrifuged immediately after collection.
Serum: Whole blood should be taken into a plain tube and allowed to clot for 30 minutes. The clot should be separated by centrifugation. Care should be taken to avoid haemolysis.
N.B. Refer to the section on serum samples stability in the Calculation of Results section (page 6).

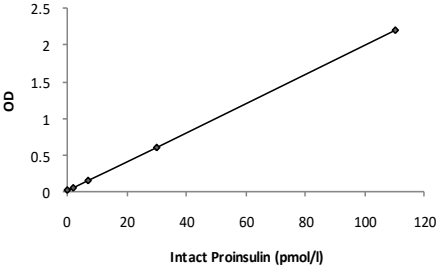
Specimen Storage

Plasma specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Assay Procedure

1. Bring all kit components and samples to room temperature before use.
2. Assemble the required number of coated strips in the plate holder. Any strips not used immediately may be resealed in the foil pouch with silica gel desiccant.
3. Pipette **50 µl Sample Buffer** into each well.
4. Pipette **50 µl each of Standard or sample** into the respective wells (standards must be run in duplicate). Attach a plate sealer and **incubate for 2 hours** at room temperature.
5. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer (300 µl each cycle) using an automatic plate washer.
6. Pipette **100 µl Biotin Conjugated Antibody** into each well. Attach a plate sealer and **incubate for 1 hour** at room temperature.
7. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer using an automatic plate washer.
8. Pipette **100 µl HRP Labelled Streptavidin** into each well. Attach a plate sealer and **incubate for 30 minutes** at room temperature.
9. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer (300 µl each cycle) using an automatic plate washer.
10. Pipette **100 µl Substrate Solution** into each well (be sure not to cross contaminate substrate with HRP Labelled Streptavidin). Place the plate in the dark and **incubate for 15 minutes** at room temperature.
11. Pipette **100 µl Stop Solution** into each well.
12. **Read absorbance** using a microplate reader set to 450nm, and, if available, with the optical density normalised by subtraction of the OD at 620/650nm.

Typical Standard Curve



N.B. This curve is for illustration only and must not be used for result calculation.

Calculation of Results

The results may be calculated automatically using a cubic spline curve fit. Other data reduction functions may give slightly different results. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard should be further diluted. For the calculation of the concentrations this dilution factor must be taken into account.

Expected Values

It is strongly recommended that each laboratory determines its own normal and abnormal values.

Studies performed with the Invitron MLT™ Intact Proinsulin Kit have demonstrated the utility of Intact Proinsulin as a marker for insulin resistance. Samples from patients with type 2 diabetes on oral medication or dietary treatment were collected from 149 sites that participated in the IRIS-II study [(1)]. In total, 2,146 male and 2,124 female patients with type 2 diabetes without insulin therapy participated in the study. In an additional study 10 groups of 50 patients, each with incremental homeostasis model assessment (HOMA) scores, were randomly chosen out of a 4,265-person cohort in order to investigate intact proinsulin and adiponectin over a wide range of insulin resistance [(2)]. Determinations of fasting values of intact proinsulin, insulin, resistin, adiponectin, and glucose were performed. The results of these studies showed that a fasting intact proinsulin concentration of >10 pmol/l predicts the presence of insulin resistance in patients with type 2 diabetes mellitus at a very high specificity and high sensitivity. Fasting proinsulin levels in normal subjects were found to be <10 pmol/l. Based on these studies, a fasting plasma concentration <10 pmol/l is considered normal while a concentration >10 pmol/l is suggestive of insulin resistance.

N.B. These studies were conducted prior to WHO International Reference Reagent IRR 84/611 being replaced by IRR 09/296. Based on the value assignment of the new preparation, samples measured against IRR 09/296 read 72.5% of the value measured against 84/611. The equivalent fasting plasma concentration in normal subjects when measured against the new reference reagent (IRR 09/296) used in this kit would be <7.25 pmol/l.

Serum samples

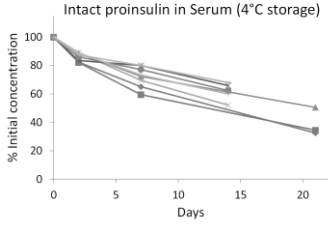
Invitron recommend using heparin or EDTA Plasma for intact proinsulin measurements. Full recovery of Intact proinsulin is not achieved from serum.

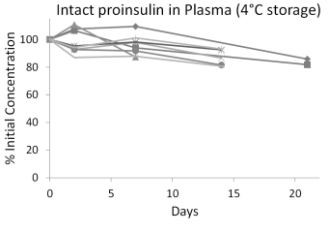
The following results were obtained on measurements of 8 serum and plasma samples, collected from patients at the same time.

Measured Intact Proinsulin (pmol/l)		
Sample	Serum	Plasma
1	19.9	21.5
2	18.6	21.2
3	61.4	70.1
4	18.4	21.8
5	15.1	19.4
6	6.5	6.5
7	10.1	12.5
8	23.5	25.2

The following results were obtained on measurements 8 serum and 8 plasma samples, stored at 4ºC.

Intact Proinsulin, % of initial concentration (mean)		
Day	Serum (%)	Plasma (%)
0	100	100
2	86	98
7	72	96
14	62	87
21	39	84





Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices, microplate reader, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor.

Interfering Substances

Interferences were studied in accordance with CLSI recommendations (EP7-A2). To study the effect of lipaemia, haemolysis and icterus, test pools were prepared by spiking plasma samples with a commercial lipid emulsion (intralipid), haemoglobin and bilirubin.
No interference due to intralipid was observed up to a concentration of 24.4 mg/mL. Interference due to haemoglobin was not apparent up to 20g/L. Bilirubin produced no apparent interference up to a concentration of 257 µmol/L.

Performance Characteristics

Sensitivity

Analytical sensitivity was estimated as the value measuring two standard deviations above the zero standard (Standard 1) and represents the lowest measurable analyte concentration that can be distinguished from zero. Calculated in this way, the limit of detection (LOD) of the assay is 0.36 pmol/l.

Precision

A study was performed using 2 control samples assayed in 20 individual assays in duplicate. The following results were obtained:

Within Run Precision

Intact Proinsulin (pmol/l)	SD (pmol/l)	CV (%)
3.8	0.19	4.9
70	1.84	2.6

Between Run Precision

Intact Proinsulin (pmol/l)	SD (pmol/l)	CV (%)
3.8	0.33	8.8
70	3.82	5.5

High Dose Hook Effect

There is no high-dose hook effect up to 10,000 pg/mL. High dose hook would not be expected to occur due to the assay architecture, which employs separate incubations with solid phase and labelled antibodies.

Linearity

Five patient samples containing elevated proinsulin concentrations were diluted in Sample Diluent Buffer. The following table shows the measured intact proinsulin concentrations of the undiluted and diluted specimens.

Measured Intact Proinsulin (pmol/l)					
Dilution Factor	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
0	69.6	56.4	18.7	26.2	6.8
1:2	38.3	27.4	10.1	14.5	3.6
1:4	18.9	13.7	5.6	7.1	1.7
1:8	10.0	7.0	2.7	3.6	0.9
1:16	5.2	3.7	1.3	2.0	0.5

Cross Reactivity

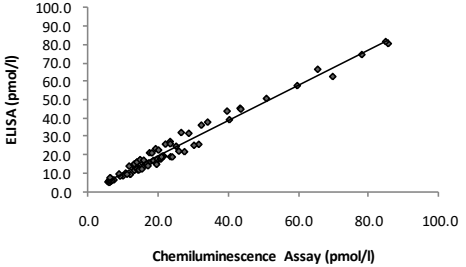
Cross reactivities of related proteins were investigated at concentrations of 100 pmol/l. Results are expressed as percentages of the reactivity of an identical concentration of intact proinsulin.

Peptide	**CR (%)**
Intact proinsulin	100
Insulin	0.0
C-peptide	0.0
32-33 split proinsulin	5.6
Des 31-32 split proinsulin	1.4
65-66 split proinsulin*	37
Des 64-65 split proinsulin*	63

** Studies have shown that 65-66 split proinsulin and Des 64-65 split proinsulin are not present at detectable levels in human samples (3)*

Correlation Data

116 patient samples covering the range 5 to 81 pmol/l were measured using the Invitron Intact Proinsulin ELISA and the Invitron Intact Proinsulin Chemiluminescence Assay. A correlation coefficient (R^2) of 0.98 was obtained, indicating close agreement between the two methods.



Limitations

- The values obtained from this assay are intended to aid in diagnosis only. As with all serological tests, interpretation of results obtained with this test must be used in conjunction with the patient's clinical symptoms, medical history and other clinical and/or laboratory findings.
- Only if test instructions are rigidly followed will optimum results be achieved.
- Use fresh plasma or specimens frozen and thawed no more than twice. Specimens that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with Wash Buffer, and that there are no residues in the wells.

References

(1) Pfutzner A et al. IRIS 2 Study: Intact proinsulin is confirmed as a highly specific indicator for insulin resistance in a large cross-sectional study design. Diabetes Technology & Therapeutics 2005; 7: 478-486.

(2) Matthias R. et al. Sensitivity and Specificity of Intact proinsulin Adiponectin and the Proinsulin/Adiponectin Ration as Markers for Insulin Resistance. Diabetes Technology & Therapeutics 2004; 6: 836-843.

(3) Sobey W et al. Sensitive and specific two-site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32-33 split proinsulins. Biochem J 1989; 260: 535-541.

For ordering information, please contact:

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