invitron
high sensitivity diagnostics

IV2-102E

English

Invitron Intact Proinsulin ELISA Kit

For in-vitro diagnostic use

CE

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Definitions

	Instructions for use
REF	Catalogue number
	Use by
LOT	Lot/Batch Code
	Storage temperature limitations
IVD	In vitro diagnostic medical device
	Manufactured by
	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 microtitre wells and a soluble antibody labelled with biotin. The sample is incubated in the microtitre 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 Microtitre Plate
  (12 x 8 wells) coated with a specific monoclonal antibody.
- Biotin Conjugated Antibody
  Biotin labelled antibody in phosphate buffer. Supplied ready to use.
- HRP Labelled Streptavidin
  HRP enzyme labelled streptavidin. Supplied ready to use.
- Standards
  (5 x 1ml lyophilized) of 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. *The standards are calibrated against WHO 1st International Standard for Proinsulin (IRP 84/611).*

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

Material required but not supplied

- Deionised 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.
- Optimal test results are only obtained when using calibrated pipettes and luminometer.
- 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. Microtitre 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 deionised 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 deionised 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. ***If serum samples are used refer to the notes on serum samples in the Calculation of Results section (page 8).***

Specimen Storage

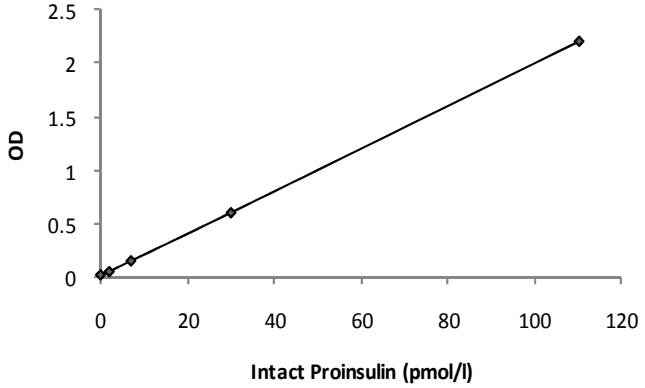
Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

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 30 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

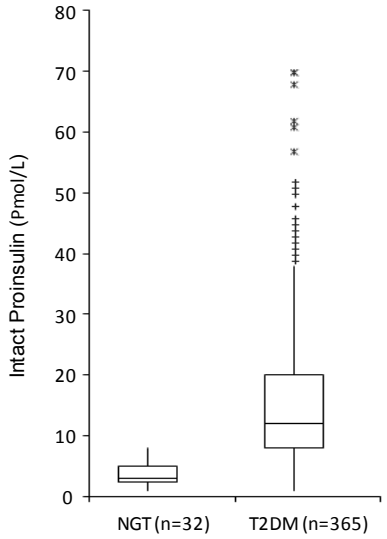
This curve is for illustration only and must not be used for result calculation.



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 has to be taken into account.

Expected Values



Fasting Intact proinsulin was measured in 365 newly diagnosed Type 2 Diabetics (T2DM) and in 32 subjects with normal glucose tolerance (NGT).

For T2DM:
Mean Intact proinsulin (pmol/l): 16.0
(n = 365 samples)

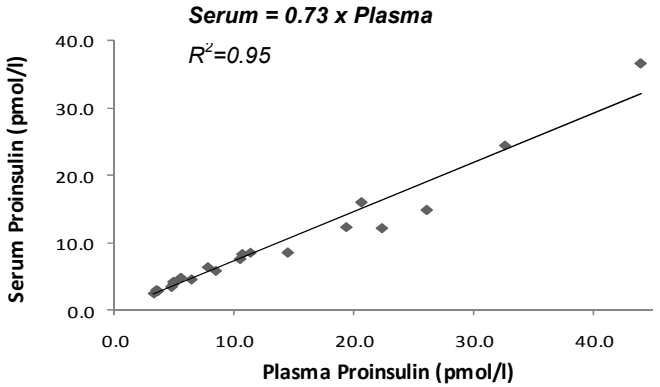
For NGT:
Mean Intact proinsulin (pmol/l): 3.8
(n = 32 samples)

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

Studies have been performed with the Invitron Intact Proinsulin Kit with adult males and females that had been diagnosed as having type 2 diabetes previously and were being treated with oral anti-diabetes drugs (1-3). Samples from patients with type 2 diabetes with oral medication or dietary treatment were collected from 149 sites that participated in the IRIS-II study. 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. Another study evaluated 48 patients with type 2 diabetes and on oral anti-diabetic treatment. Twenty women and 28 men, aged 60 (± 9 years), were studied by means of an intravenous glucose tolerance test. 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.

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 from a study performed using 20 serum and plasma samples collected from patients at the same time. A regression analysis for plasma/serum gave the following results:



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 (CLSI EP7-A2). To study the effect of lipaemia, test pools were prepared by spiking plasma samples with a commercial lipid emulsion (Intralipid Sigma). Test samples for investigating the effect of haemolysis were obtained by osmotic shock. Icteric samples were prepared by spiking plasma samples with commercial bilirubin (Sigma).
No effect of lipaemia was observed at a lipaemic index up to 975. Interference due to haemolysis was not apparent at a haemolysis index up to 467. Bilirubin produced no apparent interference up to an icterus index of 1065.

Performance Characteristics

Sensitivity

Sensitivity was estimated as two standard deviations from the mean of 4 replicates of a zero standard in 3 separate assays.
Sensitivity: 0.3 pmol/l at 95% confidence

Intra-Assay Variation

A study was performed using 2 control samples assayed in 3 individual assays in duplicate. The mean CV of the duplicates for these results is shown in the following table.

Intact Proinsulin (pmol/l)	CV%	n
3.5	4.9	3
54	5.3	3

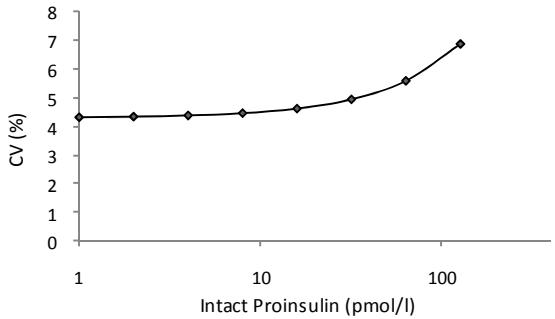
Inter-Assay Variation

A study was performed using 2 control samples assayed in 3 individual assays in duplicate in each assay to yield the following results.

Intact Proinsulin (pmol/l)	CV%	n
3.5	11.9	3
54	13.8	3

Precision profile

A precision profile was created from results of duplicate measurements of 116 patient samples from 3 assays.



High Dose Hook Effect

Because of the assay architecture, which employs separate incubations with solid phase and labelled antibodies, no high dose hook effect is experienced.

Recovery

Five plasma samples containing low endogenous intact proinsulin were spiked with recombinant proinsulin at 3 levels. Recoveries are shown as percentages of the expected result for samples within the range of 9 to 22 pmol/l.

Sample	1	2	3	4	5
Spike 5%	102.4	107.5	100.4	98.8	97.6
Spike 10%	105.1	107.1	102.8	101.9	96.1
Spike 15%	104.4	107.5	102.1	101.3	100.4

Mean spiking recovery was 102.4%.

Linearity

Four 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 proinsulin (pmol/l)				
Dilution Factor	Sample 1	Sample 2	Sample 3	Sample 4
0	46.1	46.7	22.8	48.6
1:2	24.1	26.6	12.6	27.5
1:4	11.0	12.7	6.3	12.4
1:8	5.4	5.3	3.3	6.0

Cross Reactivity

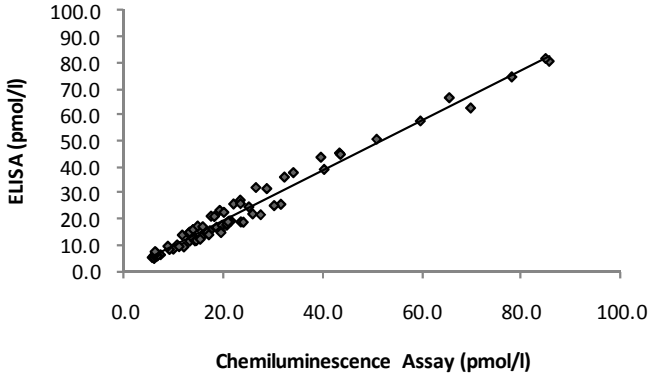
Cross reactivities of related proteins were investigated at concentrations of 100 pmol/l. Results are expressed as percentages of the reactivity of an identical concentration of intact proinsulin.

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 (4)*

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 Washing Solution, and that there are no residues in the wells.

For additional information and product support please contact:

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