invitron
high sensitivity diagnostics

IV2-002

English

MLT™ Intact Proinsulin Kit

For in-vitro diagnostic use only

MLT™ *(Molecular Light Technology)* Chemiluminescence assay for the measurement of Intact Proinsulin in human samples.

CE

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Definitions

Instructions for use

REF

Catalogue number

Use by

LOT

Batch Code

Storage temperature limitations

IVD

In vitro diagnostic medical device

Manufactured by

Contains sufficient for <N> tests

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MLT Intact Proinsulin Kit

Intended Use

Invitron's MLT Intact Proinsulin Assay is an immunometric assay using Molecular Light Technology Chemiluminescence for the quantitative measurement of intact proinsulin in human plasma 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 MLT Intact Proinsulin Assay is a two-site immunoassay, employing a specific solid phase antibody immobilised on microtitre wells and a soluble antibody labelled with a chemiluminescent acridinium ester. The sample is incubated in the microtitre well together with a buffer and, after a wash step, the labelled antibody solution is added. A second incubation is followed by a further wash step to remove unbound labelled antibody before measurement. The bound luminescence is quantified by a microtitre plate luminometer capable of *in situ* reagent addition. The luminescent reaction is a rapid flash type (>95% complete in 1 second) which permits the entire plate to be read in approximately 5 minutes.

Materials Provided

- Coated Microplate
  (5 x 96 well microplates) Stripwells coated with a specific monoclonal antibody.
  The microplate is sealed inside a foil pouch with a dessicant to maintain a moisture-free environment.
- Labelled Antibody Concentrate
  (1 x 5.5ml) Chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide.
- Labelled Antibody Diluent
  (5 x 14.1ml) Ready to use for diluting the labelled antibody to its working strength. Protein matrix including preservatives and 0.05% sodium azide.
- Standard 1 - 5
  (5 sets) 1ml lyophilized of 5 concentrations – (typically) 0.0, 1.5, 5.0, 25.0, 110 pmol/l – Recombinant intact proinsulin in a buffer matrix, lyophilized and sealed under vacuum for stability. See label for each lot of kits for actual concentrations. ***The standards are calibrated against WHO 1st International Standard for Proinsulin (IRP 84/611).***
- Controls A - B
  (2 sets) 1ml lyophilized of 2 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
  (5 x 10ml) Ready to use for sample dilution. Protein matrix including preservatives and 0.05% sodium azide.
- Wash Buffer Concentrate
  (2 x 50ml) phosphate buffered saline containing detergent and preservative.
- Product Insert
- Plate sealers – 10 each

Materials Required But Not Provided

- Detection reagents. Invitron Cat. No. IV1-001.
- Deionised water
- Microtitre plate Luminometer capable of direct injection and of measuring flash kinetics.
- Microplate washer
- Incubator (37°C)
- Calibrated Precision Micropipettes with disposable tips.

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 two-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 microplates are stable for two months if stored as described above. Reconstituted/diluted reagents are stable for 2 weeks when stored at 2-8°C.

Standards and Controls

Reconstitute each of the standards and controls by the addition of 1 ml of deionised water. Allow these to stand for 5 minutes, then mix gently to ensure all solids are dissolved. Stability of the reconstituted Standards and controls is two (2) weeks when stored at 2-8°C.

Labelled Antibody Concentrate

Pipette 900 µL of labelled antibody concentrate into one bottle of Labelled Antibody Diluent and mix thoroughly. Diluted Labelled Antibody is stable for two (2) 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.

Luminometer Set-up

The microtitre plate luminometer must be fitted with 2 injectors and it is important to check that the instrument is capable of measuring "flash" type kinetics. The measurement protocol should be set as follows:

1. Set injector 1 to deliver 100 µl of Detection Reagent 1
2. Set injector 2 to deliver 100 µl of Detection Reagent 2
3. Set a delay of 2 seconds between injection 1 and injection 2.
4. Light measurement must start at the time of the second injection (i.e. there is no delay between injection 2 and measurement).
5. Measurement time is 1 second.

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 7).

Specimen Storage

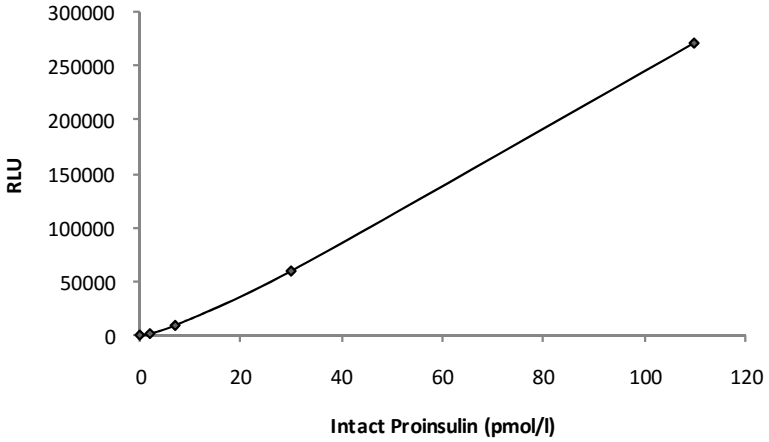
Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

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.
3. Pipette **50 µl Sample Buffer** into each well.
4. Pipette **50 µl each of Standard, control or sample** into the respective wells. Standards and controls must be run in duplicate.
5. Attach the plate sealer and incubate for **2 hours at 37ºC.**
6. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer (300 µl each cycle) using an automatic plate washer.
7. Pipette **100 µl labelled antibody** solution into each well.
8. Attach the plate sealer and incubate for a further **1 hr at 37ºC.**
9. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer using an automatic plate washer.
10. Measure the light output from each well in a plate luminometer within 15 minutes.

Typical Standard Curve

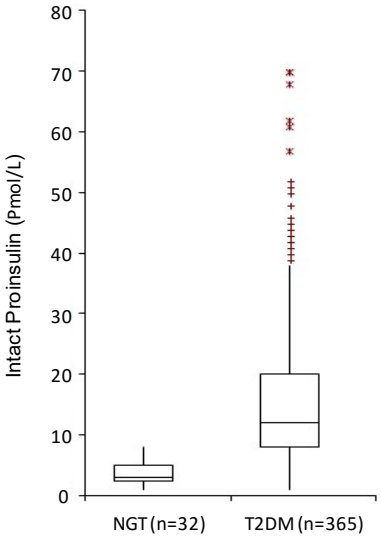
This curve is for illustration only and must not be used for result calculation.
RLU = Relative Light Units.



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).

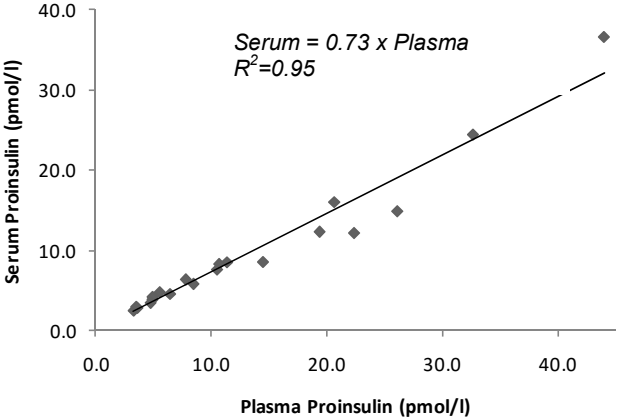
For T2DM:
Mean Intact proinsulin (pmol/l): 16.0
(n = 365 samples)

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 $\geq$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 $\geq$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; luminometer, 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 directly.

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 washing, check that all wells are filled evenly with wash buffer, and that there are no residues in the wells.
- Instructions for using appropriate luminometers are to be observed. Check that the instrument has the correct measurement protocol installed.

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

Between Assay Precision

Three plasma pools were measured in duplicate in 5 individual assays. The following results were obtained.

Intact Proinsulin (pmol/l)	CV%	n
3.38	2.61	5
27.6	4.47	5
57.2	3.57	5

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 falling 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	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

Sensitivity

Sensitivity was estimated as two standard deviations from the mean of 20 replicates of a zero standard. Calculated in this way, analytical sensitivity of the Intact Proinsulin Assay is 0.02 pmolL. The dynamic range of the assay is 0.02-100 pmol/l.

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.

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

References

Pfützner A, *et al*. Fasting intact proinsulin is a highly specific predictor of insulin resistance in type 2 diabetes. Diabetes Care 2004: 27, 682-687.

Langenfeld MR, *et al*. IRIS II Study: Sensitivity and specificity of intact proinsulin, adiponectin and the proinsulin/adiponectin ratio as markers for insulin resistance. Diabetes Technology & Therapeutics 2004: 6,836-843.

Pfützner A, *et al*. IRIS II 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.

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