Invitron Intact Proinsulin Assay Kit

IV2-002/102

Rev 01

Introduction

The Invitron Intact proinsulin Assay is an immunometric assay for the quantitative measurement of intact proinsulin in human plasma samples.

Summary

Proinsulin is a precursor molecule for insulin and is synthesised 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 non-insulin dependent (type II) 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 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. This 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.

Kit Contents

- **Standards:** Recombinant intact proinsulin in a buffer matrix, lyophilised and sealed under vacuum for stability.
- **Labelled Antibody Concentrate:** Chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide.
- **Labelled Antibody Diluent:** For diluting the labelled antibody to its working strength. Protein matrix including preservatives and 0.05% sodium azide.
- **Coated Microtitre Plate:** Microtitre plate coated with a specific monoclonal antibody. The plate is sealed inside a foil pouch with a dessicant to maintain a moisture-free environment.
- **Wash Buffer Concentrate:** (x10) phosphate buffered saline containing a detergent and 0.09% sodium azide.
- **Plate sealers**
- **Product Insert**

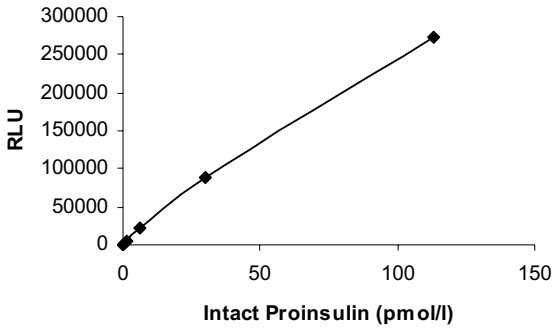
Materials Required But Not Provided

- Deionised water
- Detection reagents (Invitron Catalogue No. IV1-001)
- Uncoated strips

Procedure

1. Bring all kit components and samples to room temperature before use.
2. Reconstitute each of the standards 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.
3. Pipette 900 µl of labelled antibody concentrate into one bottle of labelled antibody diluent and mix thoroughly.
4. Make up working strength wash buffer by diluting 1 part of wash buffer concentrate with 9 parts of deionised water.
5. Assemble the required number of coated strips in the plate holder. Any strips not used immediately may be stored inside a sealed polythene bag with silica gel dessicant. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.
6. Pipette 50 µl sample buffer into each well.
7. Pipette 50 µl standard or sample into the wells. Standards must be run in duplicate.
8. Attach the plate sealer and incubate for 2 hours at 37ºC.
9. Remove the plate sealer and perform 3 wash cycles with working strength wash buffer using an automatic plate washer.
10. Pipette 100 µl labelled antibody solution into each well.
11. Attach the plate sealer and incubate for a further 1 h at 37ºC.
12. Remove the plate sealer and perform 3 wash cycles with working strength wash buffer using an automatic plate washer.
13. Measure the light output from each well in a plate luminometer.

Typical Standard Curve



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

Precision Profile

The precision of duplicate measurements was calculated for 125 patient samples. The mean coefficient of variation for these duplicates, which covered the analytical range 0.3-110 pmol/l was 5.8%.

Between Assay Precision

Intact Proinsulin (pmol/l)	CV%	n
11.8	10.3	10
21.0	8.5	10
44.4	9.9	10

Spiking 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.

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%.

Dilution Recovery

Five plasma samples were diluted with charcoal stripped human plasma by factors of 10%, 20% and 50%. Recoveries of intact proinsulin are shown as percentages of the expected result.

Sample	1	2	3	4	5
10% dilution	104.1	99.1	94.3	96.2	97.3
20% dilution	103.0	103.5	94.6	93.0	95.0
50% dilution	101.5	101.0	94.3	94.3	97.1

Mean dilution recovery was 97.9%..

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 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 pM. Results are expressed as percentages of the reactivity of an identical concentration of intact proinsulin.

Peptide	CR (%)
Intact proinsulin	100
Insulin	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
C-peptide	0.0

Range of Standards (Typically)

0-100 pmol/l

Specimen

Plasma samples should be used for the intact proinsulin assay.

Safety Precautions

1. Use good laboratory practice at all times.
2. Wear appropriate protective clothing when using this kit and when handling samples.
3. This kit contains no human-derived material.

Technical Precautions

1. Do not use components after their expiry date.
2. Once components have been opened or reconstituted, they can be used within a two-week period, provided they have been stored at 2-8ºC.
3. Do not mix reagents from different lots.
4. Take care to avoid contamination of any reagent.

This kit is intended for research use only.

For additional information and product support please contact:

Invitron Limited
Wyastone Business Park
Wyastone Leys
Monmouth NP25 3SR
United Kingdom

Tel: +44 (0)1600 891536
Fax: +44 (0)1600 891537
Email: info@invitron.co.uk
www.invitron.co.uk

Invitron Limited
Wyastone Business Park
Wyastone Leys
Monmouth NP25 3SR
United Kingdom

Tel: +44 (0)1600 891536
Fax: +44 (0)1600 891537
Email: info@invitron.co.uk
www.invitron.co.uk