Invitron Total Proinsulin Assay Kit

IV2-003/103

Rev 01

Introduction

The Invitron Total proinsulin Assay is an immunometric assay for the sum quantitation of intact proinsulin, 32-33 split proinsulinand des 31-32 split 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 proinsulin is released into the circulation, mainly as des 31-32 split proinsulin together but also with lesser amounts of 32-33 split proinsulin and intact proinsulin. It is generally believed that 65-66 split proinsulin and des 64-65 split proinsulin do not appear in detectable amounts in the circulation. Increased concentrations of circulating proinsulin may occur in non-insulin dependent (type II) diabetes and in patients with insulinoma. When used in conjunction with a specific intact proinsulin assay, measurements of total proinsulin can provide useful information on changes in the processing of insulin in such situations.

Principle

The Invitron Total 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 serum 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**

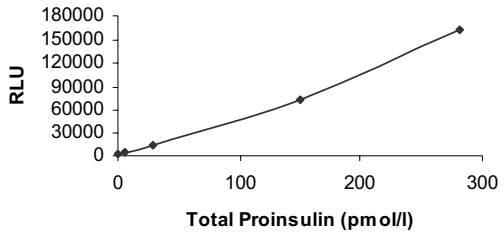
Materials Required But Not Provided

- 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 86 patient samples. The mean coefficient of variation for these duplicates, which covered the analytical range 5-235 pmol/l was 6.1%.

Between Assay Precision

Total Proinsulin (pmol/l)	CV%	n
33.8	12.8	7
81.9	13.4	7
176	6.2	7

Spiking Recovery

Four plasma samples containing low endogenous proinsulin were spiked with 49.5 and 98.0 pmol/l recombinant proinsulin (samples 1 & 4) or with 98.0 and 192 pmol/l recombinant proinsulin (samples 2 & 3). Recoveries are shown as percentages of the expected result.

Sample	1	2	3	4
1st Spike	102	104	102	94
2nd Spike	99	98	91	92

Mean spiking recovery was 97.8%.

Dilution Recovery

Five plasma samples Containing 55-160 pmol/l proinsulin were diluted with zero standard by factors of 12.5%, 25% and 50%. Recoveries of proinsulin are shown as percentages of the expected result.

Sample	1	2	3	4	5
12.5% dilution	116	100	95	93	94
25% dilution	98	111	105	96	88
50% dilution	105	115	107	100	96

Mean dilution recovery was 101.3%.

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 Total Proinsulin Assay is 0.35 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 reactivity of related proteins were investigated at concentrations of 200 pM. Results are expressed as percentages of the reactivity of an identical concentration of intact proinsulin.

Peptide	CR (%)
Intact proinsulin	100
32-33 split proinsulin	97
Des 31-32 split proinsulin	100
65-66 split proinsulin	12
Des 64-65 split proinsulin	1.0
Insulin	2.2
C-peptide	0.0

Range of Standards (Typically)

0-250 pmol/l

Specimen

Plasma samples should be used for the total 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

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Wyastone Business Park
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United Kingdom

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