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IV2-103E

English

Invitron Total Proinsulin ELISA Kit

For Research Use Only



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Definitions

 Instructions for use

 Catalogue number

 Use by

 Lot/Batch Code

 Storage temperature limitations

 Manufactured by

 Contains sufficient for <N> tests



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Intended Use

The Invitron Total Proinsulin Assay is an immunometric assay for the sum measurement of intact proinsulin, 32-33 split proinsulin and des 31-32 split proinsulin in human plasma samples.

For research use only. Not for use in diagnostic procedures.

Summary and Explanation

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 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 sample 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 immunometric, employing a specific solid phase antibody immobilised on microtitre wells and a soluble antibody labelled with horseradish peroxidase. 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. Bound enzyme label is quantified by addition of substrate to each well. After a brief incubation, the reaction is terminated by addition of a stop solution. Absorbance at 450 nm is measured in a plate reader.

Materials Provided

- **Coated Microtitre Plate -** (12 x 8 wells) stripwells coated with a specific monoclonal antibody. The plate is sealed inside a foil pouch with a desiccant to maintain a moisture-free environment.
- **Sample Buffer -** (12 ml) Ready to use. Protein matrix including preservatives.
- **HRP Conjugate Concentrate -** HRP enzyme labelled antibody (1.0ml).
- **Conjugate Diluent -** (1 x 11.0ml) Ready to use for diluting the antibody-HRP concentrate to its working strength. Protein matrix including preservatives.
- **Standards -** (5 x 1.0ml lyophilized) of 5 concentrations – (typically) 0, 5, 20, 100, 200 pmol/l – proinsulin in a buffer matrix, lyophilized and sealed under vacuum for stability. ***Standards are calibrated against the WHO 1st International Standard for Proinsulin (IRR 09/296).*** Refer to kit certificate of analysis for actual concentrations.
- **Controls -** (2 x 1.0ml lyophilized) samples containing low (A) and high (B) concentrations of 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**.
- **Substrate Solution -**Tetramethylbenzidine (TMB) substrate. Supplied ready to use.
- **Stop Solution -** ELISA stop reagent. Supplied ready to use.
- **Wash Buffer Concentrate -** Phosphate buffer including detergent (30x concentrate).
- **Product Insert**

Materials Required But Not Provided

- Deionised water
- Uncoated strips
- Plate Sealers
- Calibrated Precision Micropipettes with disposable tips.
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtitre plate reader at 450 nm (background subtraction at 620/650).

Warnings and Precautions

- For research use only. For professional use only.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet.
- 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.
- Optimal test results are only obtained when using calibrated pipettes.
- Do not mix or use components from kits with different lot numbers.
- This kit contains no human-derived material.

Sample Collection & Storage

Plasma or Serum samples. Do not use severely haemolysed specimens.

Sample Collection

Plasma: Whole blood should be collected into a tube containing EDTA or heparin anticoagulant and centrifuged immediately after collection. Plasma should be frozen and stored at -20ºC as soon as possible after separation.

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.

Sample Storage

Samples should be stored frozen at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

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.

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. Reconstituted Standards can be stored for up to two (2) weeks when stored at 2-8°C.

HRP Conjugate

Transfer the entire contents of the vial containing HRP Conjugate Concentrate (1.0ml) into the bottle of Conjugate Diluent (11.0ml) (1/12 dilution) and mix thoroughly. Working strength HRP Conjugate can be stored for up to 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. Working strength wash buffer can be stored for up to two (2) weeks at 2-8°C.

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 stored inside a sealed polythene bag with silica gel desiccant.

3. Pipette **50 µl Sample Buffer** into each well.

4. Pipette **50 µl Standard or Sample** into the respective wells. It is recommended that all standards and samples are run in duplicate.

5. Attach a plate sealer and incubate for **2 hours at Room Temperature** (18-22 ºC).

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 working strength antibody** conjugate into each well.

8. Attach a plate sealer and incubate for a further **1hr at Room Temperature** (18-22 ºC).

9. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer using an automatic plate washer.

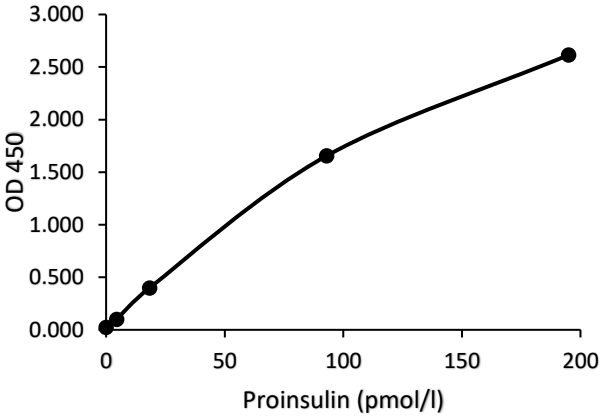
10. Add **100 µl Substrate Solution** to each well. Incubate for 15 minutes at room temperature (18-22 ºC) in the dark.

11. Add **100 µl Stop Solution** to each well.

12. Measure light transmission in a microtitre plate reader set to 450 nm and, if available, with a background subtraction measured at an OD of 620/650 nm.

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 curve fitting software (e.g. 4-parameter or cubic spline). Different 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

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

Assay Performance

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 0.5 pmol/L. The dynamic range of this assay is 0.5 – 250 pmol/L.

Within Assay Precision

Low and high controls were assayed eight times in a single run. The results are shown below.

Sample ID	Mean Concentration (pmol/l)	CV (%)
Control A	10.9	2.0
Control B	123	1.7

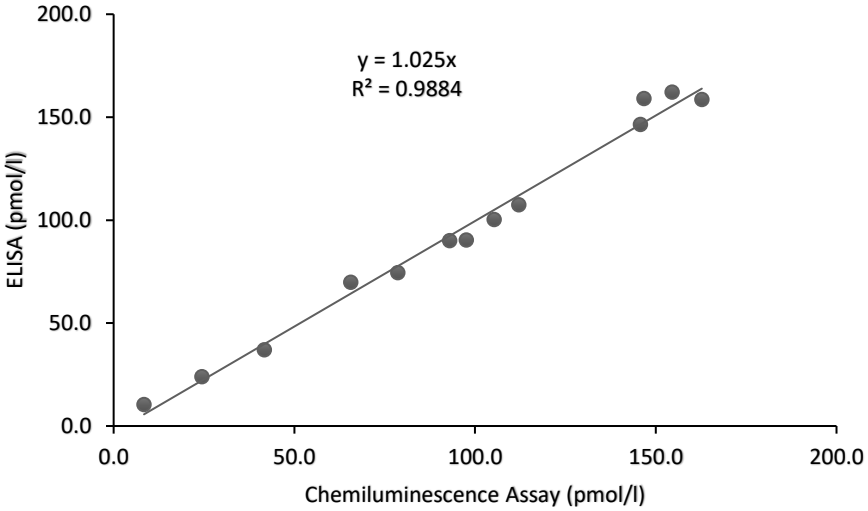
Linearity

Three plasma samples were measured in the Total Proinsulin ELISA undiluted and also diluted in assay sample buffer diluent. Results corrected for the appropriate dilution factor are given in the table below.

Dilution	Measured proinsulin (pmol/l)		
	Sample 1	Sample 2	Sample 3
0	34.7	76.3	58.4
1:2	16.5	38.6	29.1
1:4	7.1	19.1	15.3
1:8	4.3	10.9	7.1

Method Comparison

A group of samples with type 2 diabetes were measured in the Total Proinsulin ELISA and results compared with those obtained in the Invitron Total Proinsulin Chemiluminescence Assay. The results are shown below.



High Dose Hook Effect

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

Cross Reactivity

Cross reactivity with Insulin related proteins was determined by measuring each substance in the Total Proinsulin assay at a concentration of 100 pmol/l. Results are expressed as percentages of the reactivity of an identical concentration of 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

** Studies have shown that 65-66 split proinsulin and Des 64-65 split proinsulin are not present at detectable levels in human samples (Sobey W J, et al. Sensitive and specific 2-site immunoradiometric assays for human insulin, proinsulin, 65-66 split and 32-33 split proinsulins. Biochem J 1989: 260, 535-541).*

Limitations

- For Research Use Only.
- 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 is no residue in the wells.

For ordering information, please contact:

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