



Invitron Insulin Assay Kit

IV2-001/101

Introduction

The Invitron Insulin assay is an immunochemiluminometric assay for the quantitative measurement of insulin in human samples.

Summary

Insulin is a polypeptide hormone which is produced and secreted by the β -cells of the pancreas in response to a rise in circulating glucose. Its function is to facilitate the uptake of glucose into cells. Insulin measurement is useful in the investigation of hypoglycaemia, where an inappropriately high circulating concentration may be indicative of an insulin-secreting pancreatic tumour. Insulin assays are also useful in monitoring patients with insulin resistance as, for example, in non insulin-dependent (type II) diabetes.

Historically, most insulin immunoassays cross-react with the precursors of insulin biosynthesis, i.e. intact proinsulin and the split proinsulins. This product deliberately employs monoclonal antibodies which restrict this cross-reactivity to a minimum.

Principle

The Invitron Insulin Assay is a two-site immunoassay, employing an insulin-specific solid phase antibody immobilised on microtitre wells, and a soluble antibody labelled with a chemiluminescent acridinium ester. The serum or plasma sample is incubated simultaneously with the labelled antibody solution in the microtitre well, followed by a 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.

Rev 01

1

Kit Contents

- **Standards:** Lyophilised recombinant insulin in a serum matrix, calibrated against the WHO 1st International Reference Preparation 1974 (66/304), 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**

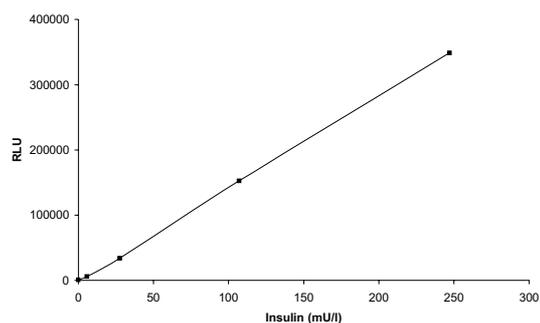
Materials Required But Not Provided

- 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 μ l 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 100 μ l labelled antibody solution into each well to be used.
7. Pipette 25 μ l of standard or sample into each well as appropriate. It is strongly recommended that samples be run in duplicate.
8. Attach the plate sealer and incubate for 2 hours at 37°C.
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.

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 3.3 to 133 mU/l (20-800 pmol/l) was 4.7%.

4

Between Assay Precision

Insulin (mU/l)	CV (%)	n
13.8	7.2	9
37.3	7.8	9
88.7	8.1	9

Spiking Recovery

5 plasma samples containing low endogenous insulin were spiked with recombinant human insulin at 3 levels. Recoveries are shown as percentages of the expected result.

Sample	1	2	3	4	5
Spike 5%	103.5	100.5	106.1	99.1	107.2
Spike 10%	99.9	106.7	106.2	101.3	107.3
Spike 15%	100.7	99.9	96.3	94.5	98.6

Mean spiking recovery was 101.9%.

5

Dilution Recovery

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

Sample	1	2	3	4	5
10% dilution	102.0	98.0	104.7	96.6	87.5
20% dilution	102.8	96.3	100.1	96.3	101.4
50% dilution	99.4	99.0	101.5	95.0	114.2

Mean dilution recovery was 99.7%.

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 Insulin Assay is 0.25 mU/l.

High Dose Hook Effect

No high dose hook effect has been observed at insulin concentrations up to 20,000 mU/l.

6

Cross Reactivity

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

Peptide	CR (%)
Insulin	100
Intact proinsulin	1.2
32-33 split proinsulin	1.6
des 31-32 split proinsulin	0.8
65-66 split proinsulin	23
des 64-65 split proinsulin	44
C-peptide	0.0

Range of Standards (Typically)

0-200 mU/l (0-1200 pmol/l)

Specimen

Both serum and plasma samples may be used.

7

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 2-8°C.
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:

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