invitron
high sensitivity diagnostics

IV2-001

English

MLT™ Insulin Kit

For in-vitro diagnostic use only

MLT™ *(Molecular Light Technology)* Chemiluminescence assay for the measurement of insulin 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 Insulin Kit

Intended Use

Invitron's MLT Insulin Assay is an immunometric assay using Molecular Light Technology Chemiluminescence for the quantitative measurement of insulin in human samples. Measurements of insulin are used in the diagnosis and management of patients with abnormalities of insulin secretion.

Summary and Explanation

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 2) diabetes.

Principle

The MLT 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.

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 5.5 ml) Chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide.
- Labelled Antibody Diluent
  (5 x 14.1 ml) Ready to use for diluting the labelled antibody to its working strength. Protein matrix including preservatives and 0.05% sodium azide.
- Standard 1 - Standard 5
  (5 sets) 1 ml lyophilised of 5 concentrations – (typically) 0.0; 6.0; 30; 110; 250 mU/l – Recombinant insulin in a serum 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 Insulin (IRP 66/304).***
- Controls A - B
  (2 sets) 1ml lyophilized of 2 samples containing low (A) and high (B) concentrations of recombinant human insulin in a buffer matrix. **Each laboratory should establish its own expected concentration range.**
- Wash Buffer Concentrate
  (1 x 50 ml) Phosphate buffered saline containing detergent and preservative.
- Plate Sealers – 5 each
- Product Insert

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 1.0ml 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

Serum, heparin plasma or EDTA plasma can be used in this assay. 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.

Specimen Storage

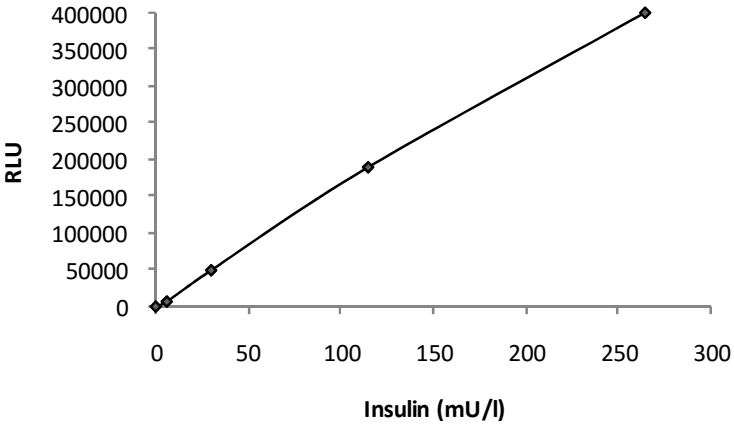
Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens held for a longer time should be stored frozen at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

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 **100 µl labelled antibody** solution into each well to be used.
4. **Pipette 25 µl of standard, control or sample** into each well as appropriate.
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 using an automatic plate washer.
7. 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 and re-assayed. Samples can be diluted in kit Standard 1. 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. Fasting values in healthy individuals are normally less than 20mU/l.

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 found up to a lipaemic index of 893. Interference due to haemolysis was not apparent at a haemolysis index up to 374. Bilirubin produced no apparent interference up to an icterus index of 352.

Performance Characteristics

Between Assay Precision

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

Insulin (mU/l)	CV%	n
14.9	7.1	5
81.3	2.4	5
145.5	7.1	5

Recovery

Five plasma samples containing low endogenous insulin were spiked with recombinant 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%.

Linearity

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

Measured insulin (mU/l)					
Dilution Factor	Sample 1	Sample 2	Sample 3	Sample 4	Sample 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 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. The dynamic range of this assay is 0.25 – 250 mU/l.

High Dose Hook Effect

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

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 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

For additional information and product support please contact:

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