invitron
high sensitivity diagnostics

IV2-101E

English

Invitron Insulin ELISA Kit

For in-vitro diagnostic use

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Definitions


Instructions for use

REF
Catalogue number


Use by

LOT
Lot/Batch Code


Storage temperature limitations

IVD
In vitro diagnostic medical device


Manufactured by

Σ
N
Contains sufficient for <N> tests

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Invitron Insulin ELISA Kit

Intended Use

The Invitron Insulin ELISA is an immunometric assay for the quantitative measurement of insulin in human plasma 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 Invitron Insulin ELISA is a two-site enzyme-linked immunosorbent assay (ELISA), employing a specific solid phase antibody immobilised on microtitre wells and a soluble antibody labelled with HRP enzyme. The sample is incubated in the microtitre well with HRP labelled antibody. After incubation, there is a wash step to remove unbound labelled antibody and substrate solution is added. Following colour development "stop reagent" is added and the colour intensity measured in a 96-well microplate reader.

Materials Provided

- **Coated Microtitre Plate**
  (12 x 8 wells) coated with a specific monoclonal antibody.
- **HRP Conjugate Concentrate**
  HRP enzyme labelled antibody (1.0ml).
- **Standards**
  (5 x 1ml lyophilized) 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**
  (2 x 1ml lyophilized) 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.***
- **Conjugate Diluent**
  Ready to use. Protein matrix including preservatives (11.0ml).
- **Substrate Solution**
  Tetramethylbenzidine (TMB) substrate. Supplied ready to use.
- **Stop Solution**
  ELISA stop solution. Supplied ready to use.
- **Wash Buffer Concentrate**
  Phosphate buffer including detergent (30x concentrate).
- **Product Insert**

Material required but not supplied

- Deionised water
- Precision pipettes and disposable tips to deliver 10-1000 µl
- Microtitre plate sealers
- A multi-channel dispenser or repeating dispenser
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtitre plate reader at 450 nm

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, they can be used within a four-week period, provided they have been stored at 2-8ºC.
- 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.

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.

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.

HRP Conjugate

Transfer the entire contents of the vial containing HRP Conjugate Concentrate (1.0ml) into the bottle of Conjugate Diluent (11.0ml) and mix thoroughly.

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.

Wash Buffer

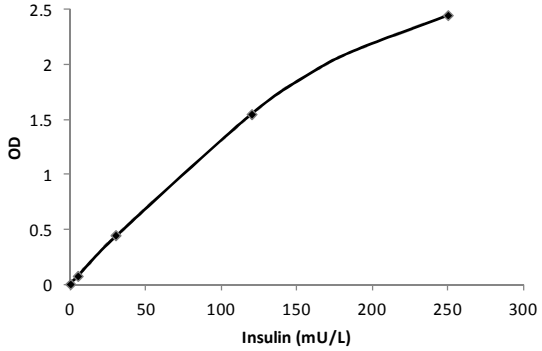
Make up working strength Wash Buffer by diluting 1 part of Wash Buffer concentrate with 29 parts of deionised water.

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 with silica gel desiccant.
3. Pipette **100 µl HRP conjugate** into each well.
4. Pipette **25 µl each of Standard or sample** into the respective wells (standards must be run in duplicate). Attach a plate sealer and **incubate for 2 hours at 37°C**.
5. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer (300 µl each cycle) using an automatic plate washer.
6. Pipette **100 µl Substrate Solution** into each well. Place the plate in the dark and **incubate for 15 minutes at room temperature**.
7. Pipette **100 µl Stop Solution** into each well.
8. **Read absorbance** using a microplate reader set to 450nm, and, if available, with the optical density normalised by subtraction of the OD at 620/650nm.

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. cubic spline or 4-parameter). Different data reduction functions may give slightly different results. The concentration of the samples can be read directly from the 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. 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, microplate reader, 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.

Performance Characteristics

Between Assay Precision

Three serum pools were measured in duplicate in 7 individual assays. The following results were obtained.

Insulin (mU/l)	CV%	n
5.0	8.3	7
19.3	6.6	7
33.5	4.7	7

Sensitivity

Assay sensitivity was calculated by constructing a precision profile of 117 samples measured in duplicate. The lower limit of quantitation (LLOQ) was calculated as the lowest dose with a coefficient of variation (CV) on duplicates of <20%.
The LLOQ of the assay is 0.9mU/l

High Dose Hook Effect

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

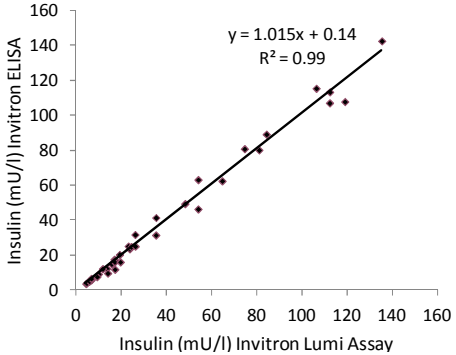
Cross Reactivity

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
Glargine	104
Aspart	94
Lispro	108
Proinsulin	1.2
C-peptide	0.0

Correlation with reference method

40 patient samples covering the range 4 to 142 mU/l were measured using the Invitron Insulin ELISA and the Invitron Insulin Chemiluminescence Assay. A correlation coefficient (R^2) of 0.99 was obtained, indicating close agreement between the two methods.



Linearity

Four samples from patients with type 2 diabetes were diluted in assay Standard 1. Measured insulin concentrations (mU/l) are shown in the table below.

Sample	1	2	3	4
Undiluted	167	57	85	88
Diluted 1/2	86.7	28.9	50.5	50.3
Diluted 1/4	46.0	15.8	26.1	25.6
Diluted 1/8	25.0	7.9	13.7	13.0

Limitations

- 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 rinsing, check that all wells are filled evenly with Wash Buffer, and that there are no residues in the wells.

For additional information and product support please contact:

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