invitron
high sensitivity diagnostics

IV2-113L

English

Invitron Glargine Luminescence Assay Kit

For in-vitro diagnostic use

CE

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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
	Contains sufficient for <N> tests

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Invitron Glargine Luminescence Assay Kit

Intended Use

The Invitron Glargine kit is an immunometric assay for the quantitative measurement of insulin glargine in human samples. Measurements of glargine are useful in monitoring diabetic patients treated with glargine.

Summary and Explanation

The long acting insulin analogue, insulin glargine is widely used in the treatment of patients with both type 1 and type 2 diabetes. Insulin glargine differs from human insulin by the presence of two additional arginine residues at the C-terminus of the B chain and the substitution of asparagine with glycine at the C-terminus of the A Chain. As a consequence of these small changes, most assays for human insulin do not detect the analogue or measure it with variable cross-reactivity. The Invitron Glargine Kit has been developed as a specific quantitative test for insulin glargine in human plasma samples.

Principle

The Invitron Glargine Assay is a two-site immunoassay, employing a monoclonal antibody immobilised on microtitre wells and a soluble antibody labelled with a chemiluminescent acridinium ester. A plasma sample is incubated in the microtitre well together 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. 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 Microtitre Plate (a)
- (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.
- Labelled Antibody Concentrate (b)
- (1 x 0.9ml) chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide.
- Labelled Antibody Diluent (c)
- (1 x 14.1ml) Ready to use for diluting the labelled antibody to its working strength. Protein matrix including preservatives and 0.05% sodium azide.
- Standards (d) - (h)
- (5 x 1.0ml lyophilized) of 5 concentrations – (typically) 0, 10, 40, 125, 250 mU/l – glargine in a serum matrix, lyophilized and sealed under vacuum for stability. See label for each lot of kits for actual concentrations.
- Wash Buffer Concentrate (IV1-005)
- (1 x 50ml) phosphate buffered saline containing detergent and preservative.
- Plate sealers – 2 each
- Product Insert

Materials Required But Not Provided

- Detection reagents. Invitron Cat. No. IV1-001.
- Deionised water
- Uncoated strips
- Microtitre plate Luminometer capable of direct injection and of measuring flash kinetics.
- 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 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 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 kits retain activity for two months if stored as described above.

Standards

Reconstitute each of the standards by the addition of 1.0 ml of deionised water. Allow these to stand for 5 minutes, then mix gently to ensure all solids are dissolved. Reconstituted standards must be stored frozen at -20ºC.

Labelled Antibody Concentrate

Transfer the entire contents of the vial containing Labelled Antibody Concentrate into the bottle of Labelled Antibody Diluent and mix thoroughly. Working strength Labelled Antibody is stable for 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. The diluted Working Wash Buffer is stable for 2 weeks at room temperature.

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

Use only EDTA Plasma. Do not use severely haemolysed specimens.

Specimen Collection

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

Specimen Storage

Specimens 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 stored inside a sealed polythene bag with silica gel desiccant. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.

3. Pipette **100 µl Standard/Sample** into each well.

4. Attach the plate sealer and incubate for **4 hours at 4ºC.**

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 working strength labelled antibody** solution into each well.

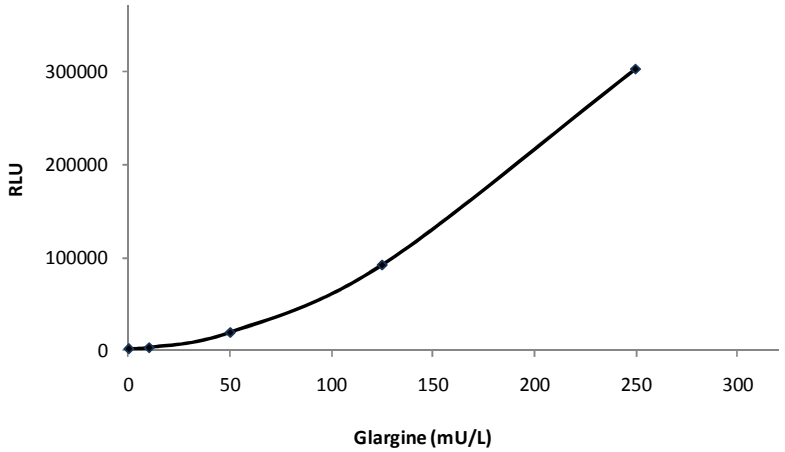
7. Attach the plate sealer and incubate for a further **24 hr at 4ºC.**

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

9. 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. 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.

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 where possible 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 Invitron directly.

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 rinsing, check that all wells are filled evenly with Washing Solution, 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.

Performance Characteristics

Cross Reactivity

Cross reactivity with Insulin and other insulin analogues was determined by measuring each substance in the glargine assay at a concentration of 250 mU/l. Results are expressed as percentages of the reactivity of an identical concentration of Glargine.

Peptide	CR (%)
Glargine	100
Insulin	2.8
Detemir	0.8
Lispro	0.2
Glulisine	2.7

Sensitivity

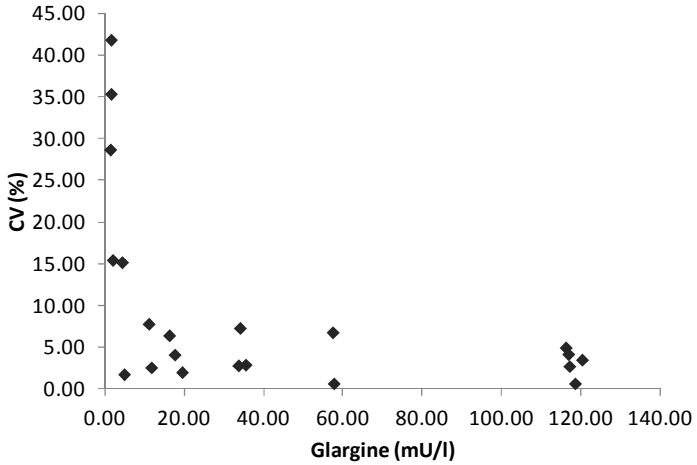
Assay sensitivity was calculated by measuring dilutions of glargine in zero standard. The lower limit of quantitation (LLOQ) was calculated as the lowest dose with a coefficient of variation (CV) on triplicates of <15%.

The LLOQ of the assay is 3.5mU/l

The dynamic range of the assay is 3.5 - 240 mU/l

Precision Profile

Assay precision was tested by measuring glargine samples over a range of concentrations. The precision profile was constructed by calculating the CV on triplicate measurements.



For additional information and product support please contact:

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