invitron
high sensitivity diagnostics

IV2-113E

English

Invitron Glargine ELISA Kit

For in-vitro diagnostic use

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Definitions

 Instructions for use

Catalogue number

 Use by

Lot/Batch Code

 Storage temperature limitations

In vitro diagnostic medical device

 Manufactured by

Contains sufficient for <N> tests

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Invitron Glargine ELISA 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 ELISA is a two-site immunoassay, employing a monoclonal antibody immobilised on microtitre wells and a soluble antibody labelled with horseradish peroxidase (HRP). A plasma sample is incubated in the microtitre well together and, after a wash step, the antibody-HRP conjugate solution is added. A second incubation is followed by a further wash step to remove unbound antibody-HRP conjugate before measurement. A substrate for the enzyme is added to each well and after a short incubation a further reagent is added to terminate the reaction. The intensity of the colour developed in each well is quantified in a microtitre plate reader set to record transmitted light at a wavelength of 450 nm.

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**

  (15 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, 10, 40, 125, 250 mU/l – glargine in a serum matrix, lyophilized and sealed under vacuum for stability. *Refer to kit certificate of analysis for actual concentrations.*

- **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
- Sealers to cover microtitre plate
- 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 *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.
- Do not mix or use components from kits with different lot numbers.
- This kit contains no human-derived material.

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.

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.

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. Use working strength HRP Conjugate within 24 hours.

Wash Buffer

Make up working strength Wash Buffer by diluting 1 part of Wash Buffer concentrate with 29 parts of deionised water. Chill working strength Wash Buffer to 2-8°C prior to use (for example refrigerate overnight). Working strength wash buffer can be stored for up to 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. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.

3. Pipette **100 µl Sample Buffer** into each well.

4. Pipette **25 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 **chilled*** 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 the plate sealer and incubate for a further **2 hr at 4ºC** (2-8 ºC).

9. Remove the plate sealer and perform **3 wash cycles** with **chilled*** 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.

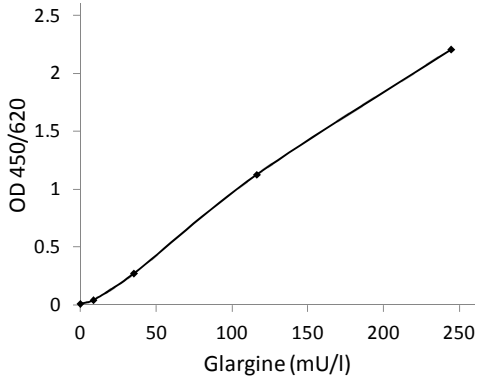
11. Add **100 µl Stop Solution** to each well.

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.

** Using wash buffer chilled to 2-8°C is not essential but will ensure optimum sensitivity.*

Typical Standard Curve

This curve is for illustration only and must not be used for result calculation.



Glargine (mU/l)	OD 450/620
0	0.015
8.7	0.040
35.4	0.250
117	1.100
245	2.300

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 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 duplicates of a zero standard in 3 separate assays.

Sensitivity: 0.8 mU/l at 95% confidence limits

Recovery
Five EDTA plasma samples were spiked with glargine at 3 levels. Recoveries are shown as percentages of the expected result.

Glargine Spike (mU/l)	Sample recovery (%)				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
15	118.1	119.0	103.1	95.6	107.7
70	111.0	110.4	105.7	102.6	94.0
140	106.6	103.5	94.0	103.5	92.8

Mean spiking recovery was 104.5%.

Linearity
Three EDTA plasma patient samples containing glargine were diluted 1:2 in Sample Buffer. The following table shows the measured glargine concentrations of the undiluted and diluted specimens.

Dilution	Measured glargine (mU/l)		
	Sample 1	Sample 2	Sample 3
0	149.3	144.9	131.6
1:2	71.0	69.8	72.3

N.B. Sample Buffer should not be used for dilutions greater than 1:2. If necessary, it is recommended that large sample dilutions are made in EDTA plasma.

High Dose Hook Effect
Because of the assay architecture, which employs separate incubations with solid phase and labelled antibodies, no high dose hook effect is experienced.

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
Human Insulin	7.5
Detemir	0
Lispro	0
Glulisine	0
Aspart	0

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 additional information and product support please contact:

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