Glargine ELISA Kit

Enzyme Immunoassay for the quantification of Glargine in human plasma (EDTA)

Catalog number: ARG82312

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Insulin glargine, marketed under the names Lantus among others, is a long-acting insulin, used in the management of type I and type II diabetes. It is typically the recommended long acting insulin in the United Kingdom. It is used once a day as an injection just under the skin. Effects generally begin an hour after use.

Common side effects include low blood sugar, problems at the site of injection, itchiness, and weight gain. Other serious side effects include low blood potassium. NPH insulin rather than insulin glargine is generally preferred in pregnancy. After injection microcrystals slowly release insulin for about 24 hours. This insulin causes body tissues to absorb glucose from the blood and decreases glucose production by the liver.

Insulin glargine was approved for medical use in the United States in 2000. In 2017, it was the 33rd most commonly prescribed medication in the United States with more than twenty million prescriptions. Measurements of glargine are useful in monitoring diabetic patients treated with glargine. [Provided from Wikipedia: Insulin glargine]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Glargine present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Glargine bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Glargine in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standards 1-5 (0, 10, 40, 125, 250 mU/L)	5 vials (Lyophilized)	4°C
HRP-Conjugated Antibody Concentrate	1 ml	4°C
Antibody Diluent	11 ml (Ready-to-use)	4°C
Sample Buffer	15 ml (Ready-to-use)	4°C
30X Wash buffer	40 ml	4°C
TMB substrate reagent	1 vials (Ready-to-use)	4°C (Protect from light)
STOP solution	1 vials (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-650 nm as the reference wave length)
- Sealers to cover microtitre plate
- Pipettes and pipette tips
- Vortex-Mixer
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. Opened reagents must be stored at 2-8 °C. Microtiter 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 8 weeks if stored as described above.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 30X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Bring all reagents and required number of strips to room temperature prior to use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents must be mixed without foaming before use.

- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Once the test has been started, all steps should be completed without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C as soon as possible. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.
b) Samples containing sodium azide should not be used in the assay.
c) Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying. Specimens held for a longer time (at least one year) should be frozen only once at-20°C prior to assay.
d) Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 30X Wash buffer into distilled water to yield 1X Wash buffer. E.g. Add 20 ml of 30 X Wash buffer into 580 ml of distilled water to a final volume of 600 ml. The diluted 1X Wash buffer is stable for 2 weeks at room temperature.
- Standards: Reconstitute each standard by the adding 1.0 ml of distilled water. Allow it to stand for 5 minutes, then mix gently to ensure all solids are dissolved completely. Reconstituted standards must be stored frozen at -20^{o}C.
- 1X HRP-Antibody conjugate: Dilute HRP-antibody conjugate concentrate (12X) into Antibody Diluent buffer to yield 1X detection antibody solution. (e.g. 500 µl of HRP-antibody conjugate concentrate (12X) + 5.5 ml of Antibody Diluent buffer) The diluted 1X HRP-antibody conjugate working solution should be used within 24 hours.
- Sample: If the initial assay found samples contain Glargine higher than the highest standard, the samples can be further diluted with Glargine negative EDTA plasma and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 18-22°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. (Optional: It is suggested to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.
2. Add **100 µl** of **Sample buffer** into each well.
3. Add **25 µl** of **standards and samples** in duplicate into wells.
4. Cover the plate and incubate for **120 minutes at RT.**
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **cold 1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. (note: the cold Wash Buffer is not essential but will ensure optimum sensitivity
6. Add **100 µl** of **1X HRP-antibody conjugate** to each well. Cover wells and incubate for **120 mins at 4°C.**
7. Aspirate each well and wash as step 5.
8. Add **100 µl** of **TMB substrate reagent** to each well. Incubate for **15 minutes at room temperature (18-22°C) in dark.**
9. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.

10. Read the OD with a microplate reader at 450 nm immediately. (optional: read at 620-650 nm as the reference wave length)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

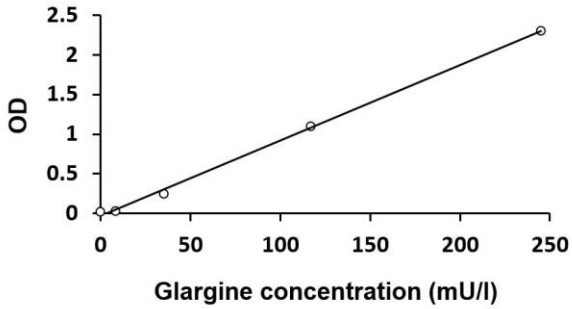
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 250 mU/L. For the calculation of the concentrations this dilution factor has to be taken into account.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Glargine ranged from 0-250 mU/L. The mean MDD was 0.8 mU /L.

Specificity

Glargine (100%)

Human Insulin (7.5%)

Detemir (0%)

Lispro (0%)

Glulisine (0%)

Aspart (0%)

Recovery

92.8-119%

Linearity

69.8-149.3%