






# KRIBIOLISA® Insulin Glargine ELISA

**REF:** KBI2001

Ver 1.2

**RUO**

Immunoassay for quantitative determination of Insulin Glargine in human serum and plasma

<b>RUO</b>	<b>For Research Use Only</b>	<b>REF</b>	<b>Catalog Number</b>
	<b>Store At</b>	<b>LOT</b>	<b>Batch Code</b>
	<b>Manufactured By</b>		<b>Biological Risk</b>
	<b>Expiry Date</b>		<b>Consult Operating Instructions</b>

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**REF** KBI2001

 96 tests

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**Introduction:**

Insulin Glargine is produced by recombinant DNA technology. It is an analogue of human insulin made by replacing the asparagine residue at position A21 of the A-chain with glycine and adding two arginine to the C-terminus (positions B31 and 32) of the B-chain. The resulting protein is soluble at pH 4 and forms micro-precipitates at physiological pH 7.4. Small amounts of insulin Glargine are slowly released from micro-precipitates giving the drug a long duration of action (up to 24 hours) and no pronounced peak concentration.

**Intended Use:**

The KRIBIOLISA® Insulin Glargine ELISA is used as an analytical tool for quantitative determination of Insulin Glargine in human serum and plasma.

**Principle:**

The method employs the quantitative sandwich enzyme immunoassay technique. In the first step, samples and standards are pipetted into microwells. If insulin Glargine is present in the samples or standards, it will form complex with Anti-insulin Glargine antibody, which is pre-coated onto microwells. In the second step, anti-Insulin Glargine biotin conjugate is pipetted and incubated. Free biotin will be removed by a washing step. In the third step, Streptavidin HRP is added and incubated. After washing TMB substrate is added to microwells and color develops proportionally to the amount of Insulin Glargine present in the samples or standards. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

Part	Description	Qty
Anti-Insulin Glargine antibody Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-Insulin Glargine antibody.	1 x 96 wells
Recombinant Human Insulin Glargine Standard	Recombinant Human Insulin Glargine Standard Concentrated	1 vial
Anti-Insulin Biotin Antibody	Anti-Insulin Glargine antibody with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane. ( lyophilized)	1 vial
Streptavidin HRP	Streptavidin HRP Concentrated	1 vial
(1X) Assay Diluent	With preservative thiomersol <0.01%	20ml
(1X) Standard Diluent	Buffered protein base with 1:1000 human serum and with preservative thiomersol <0.01%	10 ml
(1X) Sample Diluent	Buffered protein base with preservative thiomersol <0.01%	2 x 50 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	2N Sulfuric Acid	12 ml
Instruction Manual		1 no

**Materials to be provided by the End-User:**

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

**Storage Information:**

1. Store main kit components at 2-8°C.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. After reconstitution of standards, it has to be used immediately and cannot be stored.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Specimen Collection and Handling:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

**Preparation Before Use:**

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation – Serum and plasma Samples have to be diluted 1 in 1000 (v/v), e.g. 1 µl sample in 999ul of (1X) Sample diluent, prior to assay. The samples may be kept at 2 -8°C for up to three days. Long-term storage requires - 20°C.

**Reagent Preparation (all reagents should be diluted immediately prior to use):**

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. The Main standard Concentration is 3.64mg/ml. Add 1 ul of main standard stock to 2.64 ul of Standard Diluent to get a concentration of 1mg/ml. Further, add 1ul from 1mg/ml stock to 999 ul of standard diluent to get a concentration of 1ug/ml. Add 2.5ul from 1ug/ml and add 997.5ul of standard diluent to get a concentration of 2500pg/ml. Do further dilutions of the standards. 1X Standard Diluent serves as the zero standard (0pg/ml).

Standard Concentration	Standard Vial	Dilution Particulars
2500pg/ml	Standard No. 8	2.5ul of 1ug/ml (mid stock) + 997.5ul of standard Diluent (1X)
1250pg/ml	Standard No.7	500 ul Standard 8 + 500 ul of standard Diluent (1X)
625 pg/ml	Standard No.6	500 ul from Standard 7+ 500 ul of standard Diluent (1X)
312.5 pg/ml	Standard No.5	500 ul from Standard 6+ 500 ul of standard Diluent (1X)
156.25 pg/ml	Standard No.4	500 ul from Standard 5+ 500 ul of standard Diluent (1X)
78.125 pg/ml	Standard No.3	500 ul from Standard 4+ 500 ul of standard Diluent (1X)
39.063 pg/ml	Standard No.2	500 ul from Standard 3+ 500 ul of standard Diluent (1X)
19.53 pg/ml	Standard No.1	500 ul from Standard 2+ 500 ul of standard Diluent (1X)
0 pg/ml	Standard No.0	Only Standard Diluent

Use the Standards immediately upon reconstitution. Discard balance standard after use. Do not store them for further experiments.

- Working Anti-Insulin Biotin Antibody Solution – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**
- Working Streptavidin HRP Solution – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**

#### Procedural Notes:

- In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
- High Dose Hook Effect may be observed in samples with very high concentrations of Insulin Glargine. High Dose Hook Effect is due to excess of antibody for very high concentrations of Insulin Glargine present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Insulin Glargine concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
- Avoid assay of Samples containing sodium azide (NaN<sub>3</sub>), as it could destroy the HRP activity resulting in under-estimation of the amount of Insulin Glargine.
- It is recommended that all Standards and Samples be assayed in duplicates.
- Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
- If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
- The plates should be read within 30 minutes after adding the Stop Solution.
- Make a work list in order to identify the location of Standards and Samples.

#### Assay Procedure:

- Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
- Pipette **100 ul** of **prepared Standards** or **Samples** into the respective wells.
- Cover the plate and incubate for 120 minutes at Room Temperature.
- Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
- Add **100 ul** of **Anti-Insulin Biotin Antibody** into each well.
- Cover the plate and incubate for 120 minutes at Room Temperature
- Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any

residue can interfere in the reading step.

8. Add **100 ul** of **Streptavidin HRP** into each well
9. Cover the plate and incubate for 30 minutes at Room Temperature
10. Add **100 ul** of **TMB Substrate** in each well.
11. Incubate the plate at Room Temperature for 15 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
12. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
13. Read the absorbance at 450 nm with a microplate reader.

**Calculation of Results:**

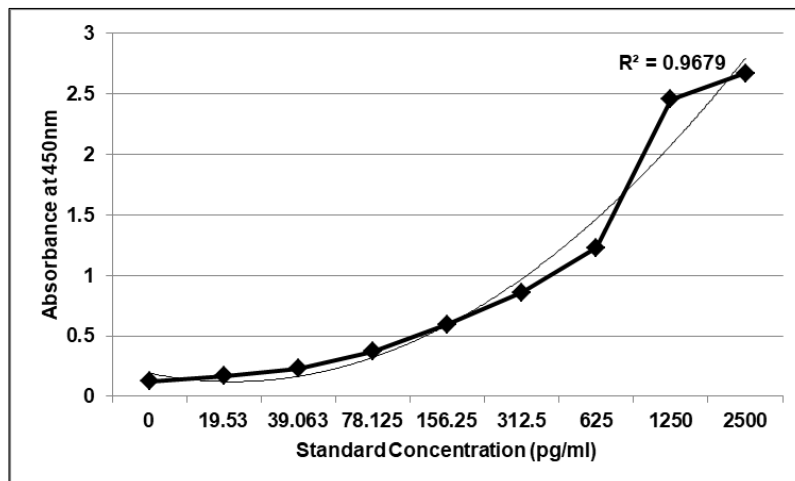
Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Using semi-log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown Human Insulin Glargine concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the Insulin Glargine concentration. If samples were diluted, multiply by the appropriate dilution factor.

Computer based curve-fitting software, 4-PL or cubic spline or polynomial regression (2<sup>nd</sup> order) may be preferred.

**Typical Data (representative only)**

<b>Std Concentration (IU/ml)</b>	<b>Mean Abs</b>	<b>Interpolated Concentration</b>	<b>% Interpolated Concentration against Actual Concentration</b>
0	0.123	--	--
19.53	0.170	18.89	94.4
39.063	0.228	34.5	101.4
78.125	0.373	131.0	167.7
156.25	0.595	228.8	146.5
312.5	0.860	334.3	107.0
625	1.227	488.7	78.2
1250	2.455	1739.2	139.1
2500	2.673	2847.1	120.1

**Typical Graph (representative only)**



Abs = absorbance at 450nm

**Quality Control:**

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

**Performance Characteristics of the Kit:**

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

**Sensitivity:**

**Limit Of Detection:** It is defined as the lowest detectable concentration and the LOD was found to be less 19.53pg/ml.

**Specificity:**

The antibodies used in the kit are monoclonal antibodies specific for Insulin Glargine. The standard / calibrator used in the kit are calibrated against commercially sourced Insulin Glargine (Lantus Injection™)

**Linearity:**

Standards provided in the kit will be used for measuring the linearity range of Insulin Glargine present in matrix.

**Precision:**

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (19.53 pg/ml), medium (312.5 pg/ml) and high (2500 pg/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results

Pool	Intra Assay %CV	Inter Assay %CV
Low	<12%	<12%
Medium	<10%	<10%
High	<10%	<10%

**Safety Precautions:**

- **This kit is For Research Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for

reagents

- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1% w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves.
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.



**References:**

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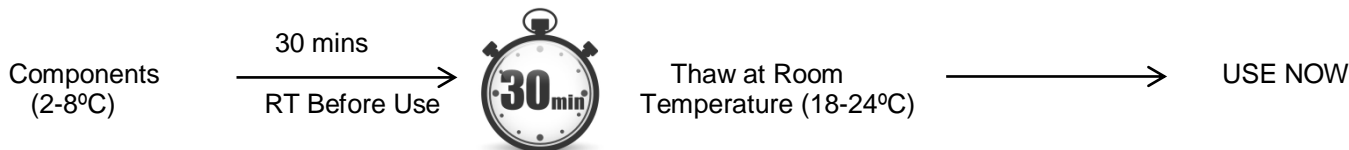
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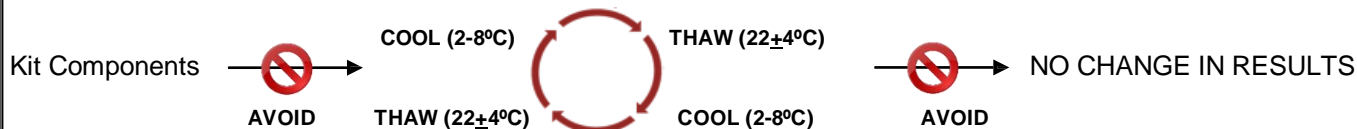
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**SCHEMATIC ASSAY PROCEDURE**

1. Remove all components, 30 minutes before adding into the assay plate.



2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.



3. Pipette **100 ul** prepared **Standards** / diluted **Samples** into each well.

4. Pipette **100 ul** prepared **Standards or Samples** into each well

5. Cover plate and incubate for at Room Temperature.

6. Aspirate and wash wells 4 times with **Wash Buffer (1X)**.

7. Pipette **100 ul Anti-Insulin Biotin Antibody Conjugate** into each well

8. Cover plate and incubate for at Room Temperature

9. Aspirate and wash wells 4 times with **Wash Buffer (1X)**

10. Pipette **100 ul Streptavidin HRP** into each well

11. Cover plate and incubate for at Room Temperature

12. Aspirate and wash wells 4 times with **Wash Buffer (1X)**

13. Pipette **100 ul TMB Substrate** into each well

14. Cover plate and incubate for at Room Temperature

15. Pipette **100 ul Stop Solution** into each well.

16. Read absorbance at 450nm with a microplate reader within of stopping reaction

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Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

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**THANK YOU FOR USING KRISHGEN PRODUCT!**



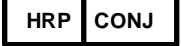



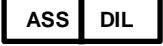








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**SYMBOLS KEY**

	Anti-Insulin Glargine antibody Coated Microtiter Plate (12x8 wells)
	Recombinant Human Insulin Glargine Standard , concentrated
	Streptavidin HRP , concentrated
	Anti-Insulin Biotin Antibody
	(1X) Sample Diluent
	(1X) Standard Diluent
	(1X) Assay Diluent
	Detection Diluent
	(20X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature

