KRIBIOLISA™ Insulin Lispro ELISA

REF : KBI2003

Ver 1.0

RUO

Enzyme Immunoassay for the quantitative determination of Insulin Lispro in serum, plasma and cell culture supernatant

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

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REF **KBI2003** **Σ 96 tests**

KRISHGEN BioSystems

Unit Nos#318/319, Shah & Nahar,
Off Dr E Moses Road, Worli, Mumbai 400018.
Tel: 91 (22) 49198700 | Email: sales@krishgen.com
http://www.krishgen.com

Introduction:

Insulin Lispro is a fast-acting insulin analog marketed by Eli Lilly as Humalog. Insulin lispro is a rapid-acting form of insulin used for the treatment of hyperglycemia caused by Type 1 and Type 2 Diabetes. Insulin is prescribed for the management of diabetes mellitus to mimic the activity of endogenously produced human insulin, a peptide hormone produced by beta cells of the pancreas that promotes glucose metabolism. Insulin Lispro is produced by recombinant DNA technology utilizing a non-pathogenic laboratory strain of Escherichia coli and was the first commercially available insulin analog.

Intended Use:

The KRIBIOLISA™ Insulin Lispro ELISA is used as an analytical tool for quantitative determination of Insulin Lispro in serum, plasma and cell culture supernatant.

Principle:

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

Materials Provided:

1. Anti-Insulin Lispro Coated Microtiter Plate (12x8 wells) - 1 no
2. Insulin Lispro Standard, (1.0 ml/vial) - 0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 ng/ml
3. Anti-Insulin Lispro Biotin Conjugate - 0.12 ml
4. HRP Conjugate - 0.12 ml
5. Sample Diluent - 4 x 50 ml
6. Wash Buffer (20X) - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

For Cell Culture Supernatant **–** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20ºC or -80ºC. Avoid repeated freeze-thaw cycles.

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 - Samples have to be diluted 1 in 400 (v/v), e.g. 5 µl sample in 2 ml sample diluent, prior to assay. The samples may be kept at 2 - 8ºC for up to three days. Long-term storage requires -20ºC.

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. To make Wash Buffer (1X); dilute 50 ml of 20X Wash Buffer in 950 ml of DI water.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Insulin Lispro.
3. It is recommended that all Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
5. 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.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at room temperature (RT).

2. Add **100 ul** of **Standards** or **Samples** into the respective wells.

3. Cover the plate and incubate for 60 minutes at room temperature, 22°C±4°C.

4. 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.

5. Pipette without delay in the same order **100 ul** of **Anti- Insulin Lispro Biotin Conjugate (1X)** into each well.

6. Cover the plate and incubate for 60 minutes at room temperature, 22°C±4°C.

7. 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. Pipette without delay in the same order **100 ul** of **HRP Conjugate (1X) into** each well.

9. Cover the plate and incubate for 60 minutes at room temperature, 22°C±4°C.

10. 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.

11. Add **100 ul** of **TMB Substrate** in each well.

12. Incubate the plate at room temperature for 15-30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

13. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

14. 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. Using Graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Insulin Lispro concentrations, find the unknown's 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 Lispro Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the highest standard

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 corresponding to a signal of Mean of '0' standard plus 2* SD.
10 replicates of '0' standards were evaluated and the LOD was found to be less than 0.2ng/ml

Linearity:
Standards provided in the kit will be used for measuring the linearity range of Insulin Lispro 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 (0.2ng/ml), medium (1.6ng/ml) and high (12.8ng/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.

Particulars	**Intra Assay**	**Inter Assay**
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

Limitations of Method

Healthy individuals should be tested negative by the Insulin Lispro. Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are suggested to consider all clinical and laboratory findings possible to state a diagnosis.

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:

… injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro.
M Lepore, S Pampanelli, C Fanelli, F Porcellati… - Diabetes, 2000 - Am Diabetes Assoc
To compare the pharmacokinetics/dynamics of the long-acting insulin analog glargine with NPH, ultralente, and continuous subcutaneous (SC) infusion of insulin lispro (continuous subcutaneous insulin infusion [CSII]), 20 C-peptide-negative type 1 diabetic patients were …

Metabolic and immunologic effects of insulin lispro in gestational diabetes.
L Jovanovic, S Ilic, DJ Pettitt, K Hugo… - Diabetes …, 1999 - Am Diabetes Assoc
OBJECTIVE: To compare the immunologic response to insulin lispro with that to regular human insulin, thereby assuring its safety for use in women with gestational diabetes, and to verify that it is effective.

Insulin lispro and regular insulin in pregnancy
A Bhattacharyya, S Brown, S Hughes, PA Vice - Qjm, 2001 - academic.oup.com
We assessed the safety of insulin lispro in gestational, type 1 and type 2 diabetes mellitus, analysing 635 pregnancies over a period of 7 years. We also evaluated patient satisfaction, sending an internationally-accepted anonymous diabetes treatment satisfaction …

diabetes insulin lisprotype 1 diabetes insulin lisproregular insulin lisproinsulin lispro patients with typesubcutaneous insulin lisproglargine insulin lisproinsulin lispro infusionglucose insulin lispro
Insulin lispro in CSII: results of a double-blind crossover study
B Zinman, H Tildesley, JL Chiasson, E Tsui, T Strack - Diabetes, 1997 - Am Diabetes Assoc
Insulin lispro is a human insulin analog that dissociates more rapidly than human regular insulin after subcutaneous injection, resulting in higher insulin levels at an earlier point in time and a shorter duration of action. The aim of the study was to evaluate if this …

A 16-week comparison of the novel insulin analog insulin glargine (HOE 901) and NPH human insulin used with insulin lispro in patients with type 1 diabetes.
P Raskin, L Klaff, R Bergenstal, JP Hallé… - Diabetes …, 2000 - Am Diabetes Assoc
OBJECTIVE: To determine the safety and efficacy of the long-acting insulin analog, insulin glargine, as a component of basal bolus therapy in patients with type 1 diabetes.

Time-action profile of inhaled insulin in comparison with subcutaneously injected insulin lispro and regular human insulin
K Rave, S Bott, L Heinemann, S Sha… - Diabetes …, 2005 - Am Diabetes Assoc
OBJECTIVE—This study compares the time-action profile of inhaled insulin (INH; Exubera) with that of subcutaneously injected insulin lispro (ILP) or regular human insulin (RHI) in healthy volunteers.

Insulin lispro
MI Wilde, D McTavish - Drugs, 1997 - Springer
Synopsis Insulin lispro, a recombinant insulin analogue, is identical to human insulin except for the transposition of proline and lysine at positions 28 and 29 in the C-terminus of the B chain. The resultant reduced capacity for self-association in solution translates into more …

Comparison of insulin aspart with buffered regular insulin and insulin lispro in continuous subcutaneous insulin infusion: a randomized study in type 1 diabetes
B Bode, R Weinstein, D Bell, J McGill, D Nadeau… - Diabetes …, 2002 - Am Diabetes Assoc
OBJECTIVE—To compare the safety and efficacy of insulin aspart (IAsp), buffered regular insulin (BR), and insulin lispro administered by continuous subcutaneous insulin infusion (CSII) in patients with type 1 diabetes. RESEARCH DESIGN AND METHODS—After …

Injection site effects on the pharmacokinetics and glucodynamics of insulin lispro and regular insulin
EW Ter Braak, JR Woodworth, R Bianchi… - Diabetes …, 1996 - Am Diabetes Assoc
OBJECTIVE The pharmacokinetics and glucodynamics of a new insulin analog, insulin lispro, and regular human insulin were compared and contrasted after subcutaneous administrations in femoral, deltoid, and abdominal injection sites.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 15 minutes before adding into the assay plate.

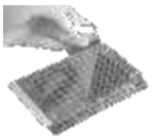
Components (2-8ºC) → 15mins RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE NOW

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

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

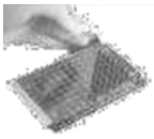
3. Pipette **100 ul Standards** / **Samples** into the respective wells

4. Cover plate  and incubate for 

at Room Temperature

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

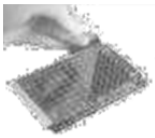
6. Pipette **100 ul Anti-Insulin Lispro Biotin conjugate** into each well

7. Cover plate  and incubate for 

at Room Temperature

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

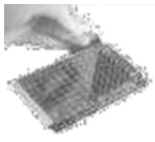
9. Pipette **100 ul Streptavidin-HRP conjugate** into each well

10. Cover plate  and incubate for 

at Room Temperature

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

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

13. Cover plate  and incubate for 

at Room Temperature

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

15. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml Insulin Lispro equivalent
1A 2A	zero std zero std			
1B 2B	0.2 ng/ml 0.2 ng/ml			
1C 2C	0.4 ng/ml 0.4 ng/ml			
1D 2D	0.8 ng/ml 0.8 ng/ml			
1E 2E	1.6 ng/ml 1.6 ng/ml			
1F 2F	3.2 ng/ml 3.2 ng/ml			
1G 2G	6.4 ng/ml 6.4 ng/ml			
1H 2H	12.8 ng/ml 12.8 ng/ml			
3A 4A	Sample			
3B 4B	Sample			

LIMITED WARRANTY

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