






KRIBIOLISA™ Anti-Lispro ELISA

REF : KBI9002

Ver 1.0

RUO

Enzyme Immunoassay for qualitative detection of antibodies to Lispro in serum.

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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 96 tests



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:

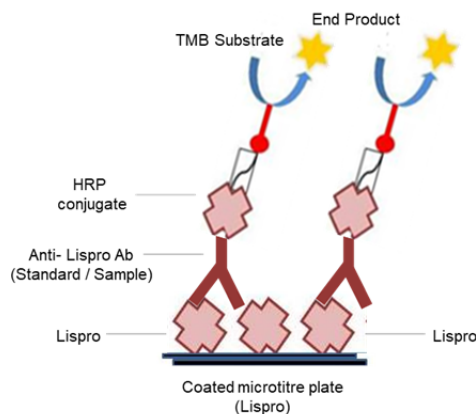
Insulin lispro, sold under the brand name **Humalog** among others, is a fast acting insulin analog. It was first approved for use in the United States in 1996, making it the first insulin analog to enter the market. Insulin lispro has one primary advantage over regular insulin for postprandial glucose control. It has a shortened delay of onset, allowing slightly more flexibility than regular insulin, which requires a longer waiting period before starting a meal after injection. Both preparations should be coupled with a longer acting insulin or an insulin infusion pump for good glycemic control.

Intended Use:

The KRIBIOLISA™ Anti-Lispro ELISA is used for qualitative detection of antibodies to Lispro in serum.

Principle:

The method employs the qualitative enzyme immunoassay technique. A commercially available Lispro is pre-coated onto microwells. Samples and Controls are pipetted into microwells and antibodies to human Lispro present in the sample and controls are bound by Lispro. In the second step, a HRP (horseradish peroxidase) conjugate is pipetted and incubated. Excess HRP conjugate will be removed by washing. Addition of TMB substrate will develop blue color and intensity of blue colour in wells are proportional to the concentration of anti- Lispro antibody present in Positive control or sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

1. Lispro Coated Microtiter Plate (12x8 wells) - 1 no
2. Positive Controls - 1 ml
3. Negative Control - 1 ml
4. Lispro:HRP Conjugate - 12 ml
5. (5X) Assay Diluent - 10 ml
6. (20X) Wash Buffer - 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 25µl to 1000µl
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 2°C to 8°C 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 (18-25 °C). 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.

Preparation Before Use:

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

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. High Dose Hook Effect may be observed in samples with very high concentrations of Anti- Lispro Antibody. High Dose Hook Effect is due to excess of very high concentrations of Anti- Lispro Antibody 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 Anti- Lispro Antibody concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Anti- Lispro Antibody.
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. 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.

7. The plates should be read within 30 minutes after adding the Stop Solution.
8. 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.
2. Pipette out **100 ul** of **Controls** and **Samples** into the respective wells as mentioned in the work list.
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 **Lispro:HRP Conjugate** 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. Add **100 ul** of **TMB Substrate** in each well.
9. 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.
10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate reader.

Interpretation of Results:

Results are interpreted qualitatively by calculating a cut-off value. The same maybe done as under –

Read the Sample and Negative Control wells on microtiter plate reader at 450nm. The OD (Optical Density) of NC (Negative Control) in triplicate should be used for calculating the mean and standard deviation. This is the Blank_{mean}. The cut-off for Positives is equal to a value greater than (Blank_{mean} + 2*Standard Deviation).

Formula:

Positive Sample Value = OD > (Blank_{mean} + 1 or 2 * SD)

1= If samples are in single wells

2= If samples are in duplicates

3= If samples are in triplicates

For Example –

Sample Type	Absorbance #1	Absorbance #2	Absorbance #3	Mean
Negative	0.200	0.219	0.221	0.213
Standard Deviation	0.200-0.213 = -0.013	0.219-0.213 = 0.006	0.221-0.213 = 0.008	

$$\text{Mean Standard Deviation} = \sqrt{(-0.013)^2 + (0.006)^2 + (0.008)^2} / n-1 = 0.0082$$

Therefore Cut-Off = Mean + 2 * SD
 = 0.213 + 2 * 0.0082
 = 0.213 + 0.0164
 = 0.229 say 0.23

Typical Reference Values would then be:

Anti- Lispro	Cut-Off Values
Negative	<=0.23
Positive	> 0.23

Note:

The standard deviation (SD) provides an estimate of the reproducibility of replicate data points and can provide confidence levels for assessing if one value is truly different from another. Whatever the measured value, a certain percentage of the values obtained are contained within the standard deviation. For instance, one SD on either side of the mean contains 68% of the values under the curve of that distribution. Approximately two SD (actually 1.96 SD) on either side of the mean contains 95% of all of the values and approximately three SD (actually 2.58 SD) contains 99% of all values. Thus if a value that is greater than three SD different from the mean of a set of samples is obtained, one can be 99% confident that it is truly different from the first set of samples. Mathematically, the SD is the square root of the sum of the variances squared divided by the number of samples minus one.

A Typical Assay Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	N.C.	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43
B	N.C.	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44
C	P.C.	S4	S8	S12	S16	S20	S24	S8	S32	S36	S40	S44
D	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45
E	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45
F	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	N.C.
G	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	P.C.
H	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43	P.C.

P.C – Positive control wells
 N.C – Negative control wells
 S. – Sample extract wells

Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

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.

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.

**LIMITED WARRANTY**

Krishgen Biosystems 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, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems 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.

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