KRIBIOLISA® Insulin Aspart ELISA

REF : KBI2002

Ver 1.0

RUO

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

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 KBI2002 Σ **96 tests**

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Introduction:

Insulin aspart is produced by recombinant DNA technology. It is a rapid-acting analogue of human insulin created by substituting the proline residue at position B28 of the B-chain with aspartic acid. This single amino-acid modification reduces the tendency of insulin molecules to self-associate into hexamers, allowing the insulin to remain predominantly in monomeric form after subcutaneous injection. As a result, insulin aspart is rapidly absorbed into the bloodstream, leading to a fast onset of action, an early peak effect, and a short duration of action. These pharmacokinetic properties make insulin aspart particularly suitable for controlling postprandial blood glucose levels.

Intended Use:

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

Principle:

The method employs the sandwich enzyme immunoassay technique. Human Canine/Porcine Insulin are pre-coated onto the microwells. Samples or standards are pipetted into the microwells, and Insulin Aspart present in the samples or standards binds to the immobilized capture antibodies. Subsequently, an HRP (horseradish peroxidase)–conjugated anti-insulin antibody is added and incubated, forming an antibody–antigen–antibody sandwich complex. After washing the microwells to remove any non-specific binding, the ready-to-use substrate 3,3',5,5'-tetramethylbenzidine (TMB) solution is added, and colour develops proportionally to the amount of Insulin Aspart present in the sample. The colour development is stopped by the addition of stop solution, and the absorbance is measured at 450 nm.

Materials Provided:

Part	Description	Qty
Human Canine/Porcine Insulin Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Human Canine/Porcine Insulin antibody.	1 x 96 wells
Recombinant Insulin Aspart Injection	Recombinant Insulin Aspart Injection Concentrated	1 vial
Human-Insulin Detection Antibody	Human Insulin detection antibody with buffered protein base with preservative thiomersol <0.01% (lyophilized)	1 vial
Streptavidin:HRP	Streptavidin:HRP Concentrated	1 vial
Assay Diluent	With preservative, thiomersol <0.01%	20 ml
(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	0.73 M phosphoric 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 2-8ºC 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. The Main standard Concentration is 3.5 mg/ml. Add 1 ul of main standard stock to 2.5 ul of Standard Diluent to get a concentration of 1 mg/ml. Further, add 1 ul from 1mg/ml stock to 999 ul of standard diluent to get a concentration of 1ug/ml. Add 1 ul from 1 ug/ml and add 999 ul of standard diluent to get a concentration of 1000 pg/ml. Do further dilutions of the standards. 1X Standard Diluent serves as the zero standard (0 pg/ml).

Standard Concentration	Standard Vial	Dilution Particulars
1000 pg/ml	Standard No.7	1 ul of 1ug/ml (mid stock) + 999 ul of standard Diluent (1X)
500 pg/ml	Standard No.6	500 ul from Standard 7 + 500 ul of standard Diluent (1X)
250 pg/ml	Standard No.5	500 ul from Standard 6 + 500 ul of standard Diluent (1X)
125 pg/ml	Standard No.4	500 ul from Standard 5 + 500 ul of standard Diluent (1X)
62.5 pg/ml	Standard No.3	500 ul from Standard 4 + 500 ul of standard Diluent (1X)
31.25 pg/ml	Standard No.2	500 ul from Standard 3 + 500 ul of standard Diluent (1X)
15.62 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.

4. **Working Human Insulin Detection Antibody Solution – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**
5. **Working Streptavidin:HRP Solution – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**

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 Insulin Aspart. High Dose Hook Effect is due to excess of antibody for very high concentrations of Insulin Aspart 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 Aspart 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 Insulin Aspart.
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. 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.

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

3. Cover the plate and incubate for 120 minutes at Room Temperature.

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. Add **100 ul** of **Human Insulin Detection Antibody** into each well.

6. Cover the plate and incubate for 120 minutes at Room Temperature.

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 **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 Aspart 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

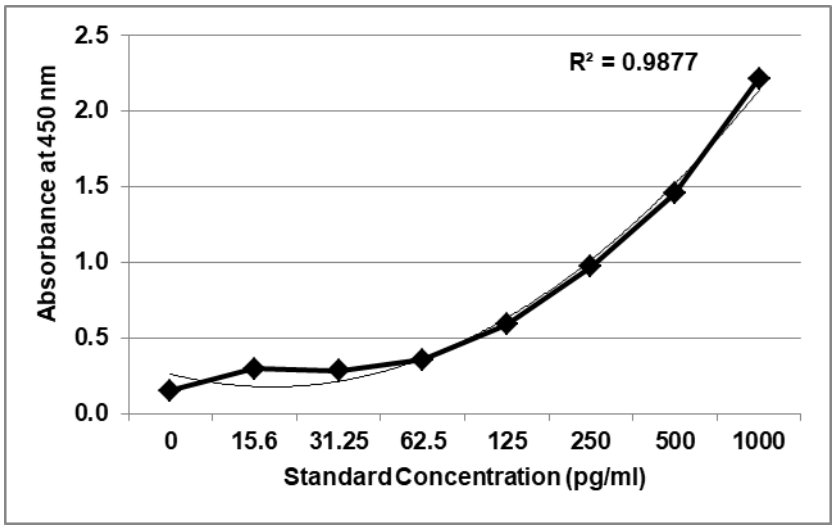
the x-axis and read the Insulin Aspart concentration. If samples were diluted, multiply by the appropriate dilution factor.

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

Typical Data (representative only)

Std Concentration (pg/ml)	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.152	--	--
15.62	0.298	32.7	94.4
31.25	0.283	28.5	91.3
62.5	0.355	49.1	78.6
125	0.590	122.8	98.3
250	0.974	265.1	106.0
500	1.455	489.6	97.9
1000	2.215	1003.0	100.3

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 than 19.53 pg/ml.

Specificity:

The capture antibody used in the kit is a highly specific anti-Insulin Aspart antibody, raised against the unique structural epitope of Insulin Aspart that distinguishes it from native human insulin and other insulin analogues. The standard used is Insulin Aspart, expressed and purified to biosimilar/research grade quality.The assay demonstrates high-affinity and selective binding to Insulin Aspart, enabling accurate quantification without significant cross-reactivity to endogenous human insulin, other insulin analogues, or unrelated serum proteins, thereby ensuring reliable and specific detection of Insulin Aspart in human serum and plasma samples.

Linearity:
Standards provided in the kit will be used for measuring the linearity range of Insulin Aspart 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.
- 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:

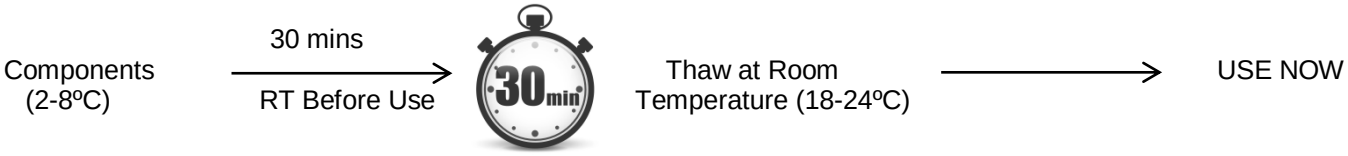
Wen WL, Tsai KB, Lin YH, Hwang SJ, Hsiao PJ, Shin SJ, Hung WW. Successful management of type IV hypersensitivity reactions to human insulin analogue with injecting mixtures of biphasic insulin aspart and dexamethasone. J Formos Med Assoc. 2019 Apr;118(4):843-848

Haahr H, Heise T. Fast-Acting Insulin Aspart: A Review of its Pharmacokinetic and Pharmacodynamic Properties and the Clinical Consequences. Clin Pharmacokinet. 2020 Feb;59(2):155-172.

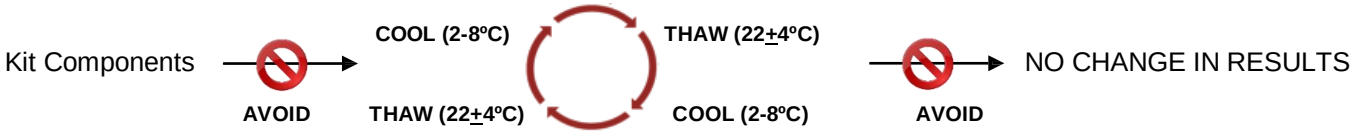
Drugs and Lactation Database (LactMed®) [Internet]. National Institute of Child Health and Human Development; Bethesda (MD): Mar 15, 2025. Insulin.

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 120 mins 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 120 mins 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 30 min 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 15 min at Room Temperature.

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

16. Read absorbance at 450nm with a microplate reader within 15 min of stopping reaction.

LIMITED WARRANTY

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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SYMBOLS KEY

MTP	Human Canine/Porcine Insulin Coated Microtiter Plate (12x8 wells)
STD	Recombinant Anti Aspart Injection, Concentrated
HRP CONJ	Streptavidin:HRP, concentrated
BIOTIN AB	Human Insulin Detection Antibody, lyophilized
1X SAMP DIL	(1X) Sample Diluent
1X STD DIL	(1X) Standard Diluent
ASY DIL	Assay Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature