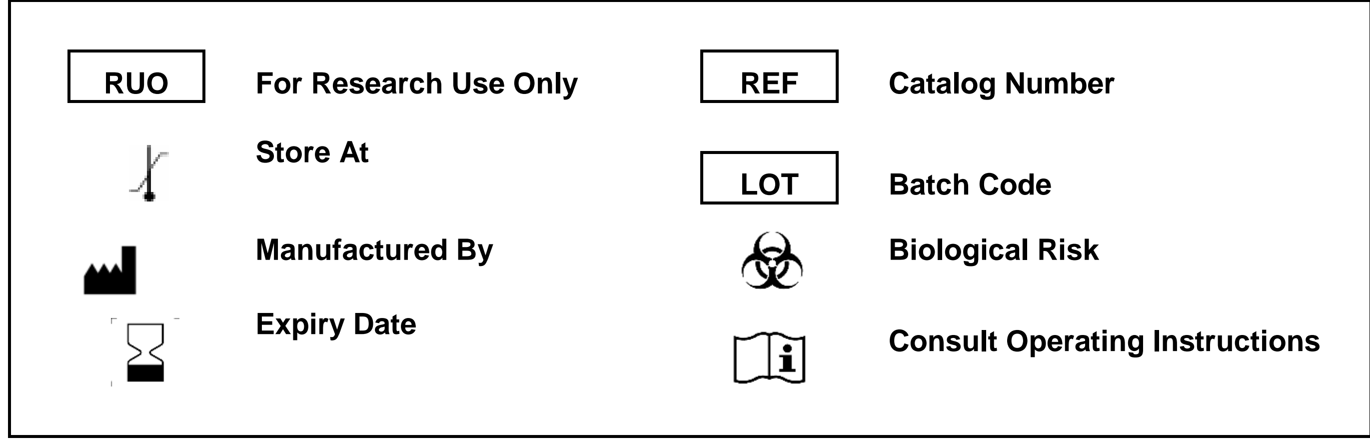
KRIBIOLISA™ Exenatide ELISA

REF : KBI5013

Ver1.1

RUO

Immunoassay for the quantitative determination of Exenatide in serum, plasma and cell culture supernatant



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Introduction:

Exenatide or Extendin-4 is a potent GLP-1 receptor agonist. Exenatide (marketed as Byetta) is one of a new class of medications (incretin mimetics) approved (in April 2005) for the treatment of diabetes mellitus type 2 (It was not approved for use in diabetes mellitus type 1).

Intended Use:

The KRIBIOLISA™ Exenatide ELISA is used as for the quantitative determination of Exenatide in serum, plasma and cell culture supernatant.

Principle:

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to Exenatide are pre-coated onto microwells. Samples and standards are pipetted into microwells and Exenatide present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-Exenatide antibody is pipetted and incubated with samples. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Exenatide in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-Exenatide Coated Microtiter Plate (96 wells) – 1 no
2. Exenatide Standards (0, 1, 3, 5, 10, 20, 30 & 50 ng/ml) – 0.5ml x 8 vials
3. Anti-Exenatide:HRP, – 12ml
4. Sample Diluent – 50ml
5. Wash Buffer (20X) – 25 ml
6. TMB Substrate – 12 ml
7. Stop Solution – 12 ml
8. 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. Deionised (DI) water
4. Wash bottle or automated microplate washer
5. Linear graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at 2-8°C. Store ready to use Standard at -20°C. Upon thawing, aliquot standards into polypropylene vials and store at -20°C as per assay requirements.
2. All the reagents and wash solutions should be used within expiry date mentioned on the kit.
3. Before using, bring all components to room temperature (18-25 ºC). 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 -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

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

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 Exenatide. High Dose Hook Effect is due to excess of antibody for very high concentrations of Exenatide 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 Exenatide 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 Exenatide.
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 duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at room temperature (RT).
2. Pipette out **100µl** of **Standards** and **Samples** into the respective wells as mentioned in the work list.
3. Cover the plate and incubate it for 60 minutes at room temperature, 20°C±4°C.
4. Aspirate and wash plate **5 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 **100µl** of **Anti-Exenatide:HRP Conjugate** into each well.

6. Cover the plate and incubate it for 60 minutes at room temperature, 20°C±4°C.
7. Aspirate and wash plate 5 times with **Wash Buffer (1X)** as in step 4.
8. Add **100µl** 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 **100µl** of **Stop Solution**. Wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate reader.

Example of a Work list

Well #	Contents	Abs at 450nm	Mean Absorbance	ng/ml Exenatide equiv.
1A 2A	Zero Std Zero Std			
1B 2B	1.0 ng/ml 1.0 ng/ml			
1C 2C	3.0 ng/ml 3.0 ng/ml			
1D 2D	5.0 ng/ml 5.0 ng/ml			
1E 2E	10.0 ng/ml 10.0 ng/ml			
1F 2F	20.0 ng/ml 20.0 ng/ml			
1G 2G	30.0 ng/ml 30.0 ng/ml			
1H 2H	50.0 ng/ml 50.0 ng/ml			
3A 4A	Sample A Sample A			

Calculation of Results:

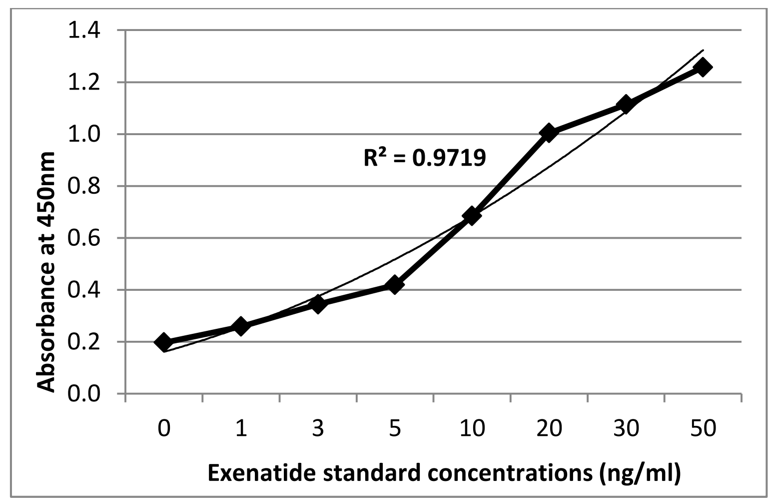
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using linear graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Exenatide 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 Exenatide 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 50 ng/ml standard.

Typical Data:

This standard curve indicated overleaf was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.



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:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Detection Range: 1ng/ml - 50ng/ml. The standard curve concentrations are 50ng/ml, 30ng/ml, 20ng/ml, 10ng/ml, 5ng/ml, 3ng/ml and 1ng/ml.

Sensitivity: The minimum detectable dose of Exenatide is typically less than 1 ng/ml .The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by subtracting two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Precision :

Intra-assay Precision: 3 samples with low, middle and high level human Exenatide were tested 20 times on one plate, respectively.

Inter-assay Precision: 3 samples with low, middle and high level human Exenatide were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/mean X 100
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Safety Precautions:

- This kit is for in vitro 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.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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