KRIBIOLISA™ Buserelin (Suprefact) ELISA

REF : KBI5003

Ver 1.0

RUO

Immunoassay for Quantitative estimation of Buserelin 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 KBI5003 Σ 96 tests

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Introduction:

Buserelin is a LHRH agonist used for the palliative treatment of hormone-dependent advanced carcinoma of the prostate gland in males and treatment of endometriosis in females. Buserelin may be used in the treatment of hormone-responsive cancers such as prostate cancer or breast cancer, estrogen-dependent conditions (such as endometriosis or uterine fibroids), and in assisted reproduction. Buserelin stimulates the pituitary glands gonadotrophin-releasing hormone receptor (GnRHR). Buserelin desensitizes the GnRH receptor, reducing the amount of gonadotropin. In males, this results in a reduction in the synthesis and release of testosterone. In females, estrogen secretion is inhibited. While initially, there is a rise in FSH and LH levels, chronic administration of Buserelin results in a sustained suppression of these hormones.

Intended Use:

The KRIBIOLISA™ Buserelin (Suprefact) ELISA kit is used for estimation of Buserelin in solutions and human serum.

Principle:

The Buserelin ELISA is a competitive immunoassay for the determination of Buserelin. The GnRH antibody are coated on 96 well plate. A constant concentration of Biotinylated GnRH and varying concentration of unlabeled Buserelin or sample compete for binding to antibodies. Captured Biotinylated GnRH are subsequently bound by Streptavidin-HRP which produces a soluble colored product after addition of TMB substrate. The enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is inversely proportional to the amount of bound Buserelin present in standards or samples.

Materials Provided:

Part	Description	Qty
Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with GnRH antibody.	1 x 96 wells
Buserelin Standard	Recombinant Buserelin standard (lyophilized; 500 pg/ml)	1 vial
Biotin Detection	Biotinylated GnRH prepared in buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane- (10ul)	1 Vial
(1X) Standard Diluent	Buffered protein base with protein stabilizer and 1:1000 human serum and preservative sodium azide < 0.1%	10ml
(1X) Sample Diluent	Buffered protein base with BSA and preservative sodium azide < 0.1%	50ml X 2
Streptavidin – HRP	Concentrated Streptavidin HRP - (420ul)	1 vial
Assay Diluent	Buffer with protein stabilizer and preservatives 0.02% methylisothiazolone and 0.02% bromonitrodioxane.	20 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	0.73M 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.
9. Incubator.

Storage Information:

1. Store kit components at 2-8°C.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date. Before using, bring all components to room temperature (18-25ºC).
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions

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.

Specimen Collection and Handling:

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 Serum & Plasma - Samples have to be **diluted 1:1000 (v/v)**, e.g. **1 ul sample + 999 ul 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. Bring all kit components and samples to room temperature (18-25°C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. To make **Wash Buffer (1X)**; dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.
3. **Standard:** Reconstitute the lyophilized standard in 250ul of Standard diluent to get a concentration of 2000pg/ml. Keep the standard for 15 minutes. 2000pg/ml is the top standard. Prepare the remaining standards as per the below table. Standard Diluent (1X) serves as the zero standard (0 ng/ml).

Standard Concentration	Standard No	Dilution Particulars
2000 pg/ml	Reconstituted standard	Lyophilized Standard provided in the Kit + 250ul of Standard Diluent (1X).
1000 pg/ml	Standard No. 7	125ul of Reconstituted standard + 125 ul of Standard Diluent (1X)
500 pg/ml	Standard No.6	125ul of Standard No. 7 + 125 ul Standard Diluent (1X)
250 pg/ml	Standard No.5	125 ul of Standard No. 6 + 125 ul Standard Diluent (1X)
125 pg/ml	Standard No.4	125 ul of Standard No. 5 + 125 ul Standard Diluent (1X)
62.5 pg/ml	Standard No.3	125 ul of Standard No. 4 + 125 ul Standard Diluent (1X)
31.25 pg/ml	Standard No.2	125 ul of Standard No. 3 + 125 ul Standard Diluent (1X)
15.6 pg/ml	Standard No.1	125 ul of Standard No. 2 + 125 ul Standard Diluent (1X)
0 pg/ml	Standard No. 0	Only Standard Diluent (1X)

4. **Biotin Detection:** The **Detection conjugation** is provided in a concentrated form. Dilute as required prior to running the assay using **Assay Diluent**. Add 1 µl of concentrated Detection Antibody to 499 µl of Assay Diluent to make Mid Stock.
   Working Solution: Add 125ul of Mid Stock in 2375ul of Assay Diluent and use this solution as working solution,
5. **Streptavidin: HRP:** The **Streptavidin: HRP** is provided in a concentrated form. Add 200ul of Conc. Streptavidin HRP in 4800ul of Assay Diluent.

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 Buserelin.
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 low / incorrect results.
6. The plates should be read within 30 minutes after adding the Stop Solution.
7. It is advisable to make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. 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 RT.

2. Pipette **50 ul** of prepared **Standards** or diluted **Samples** into the respective wells.

3. Pipette **50 ul** of **Biotinylated Detection** into each well

4. Cover the plate and incubate for 60 minutes at 37°C.

5. 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.

6. Add **100 ul** of **diluted Streptavidin:HRP Conjugate** in each well.

7. Incubate the microplate for 30 minutes at 37°C.

8. 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.

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

10. Incubate the plate at 37°C for 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.

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

12. Read the absorbance at 450 nm with a microplate reader.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate 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 Buserelin 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 Buserelin concentration. If samples were diluted, multiply by the appropriate dilution factor.

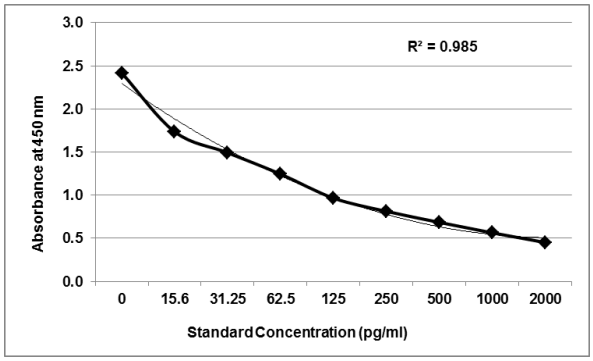
Software which is able to generate a linear regression like cubic spline or 4PL (2^{nd} order) is best recommended for automated results.

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 2000 pg/ml standard.

Typical Data

Standard Concentration (pg/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	2.417	--	--
15.6	1.736	15.4	98.5
31.25	1.493	31.1	99.4
62.5	1.246	61.3	98.0
125	0.967	140.8	112.6
250	0.814	243.3	97.3
500	0.686	429.7	85.9
1000	0.565	896.1	89.6
2000	0.453	2825.6	141.3

Typical Graph



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 15 ng/ml

Specificity:
The calibrators used are certified against commercially available Suprefact™.

Linearity:
Standards provided in the kit will be used for measuring the linearity range of Buserelin present in matrix. The standard graph range indicated is 0 pg/ml to 2000 pg/ml.

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 (15.6 ng/ml), medium (125 ng/ml) and high (2000 ng/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	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

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.

SCHEMATIC ASSAY PROCEDURE

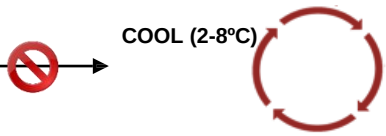
1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

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

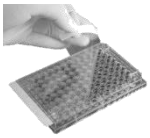
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

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

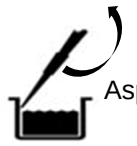


3.  Pipette **50 ul Standards / Samples** into each well.

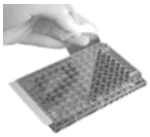
4.  Pipette **50 ul Biotinylated Detection** into the respective wells.

5. Cover plate  and incubate for 

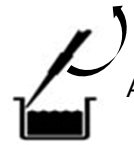
at 37°C.

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

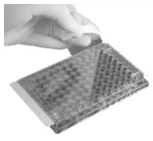
7.  Pipette **100 ul diluted Streptavidin:HRP** into each well.

8. Cover plate  and incubate for 

at 37°C.

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

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

11. Cover plate  and incubate for 

at 37°C.

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

13. 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	pg/ml Buserelin equivalent
1A 2A	0 pg/ml 0 pg/ml			
1B 2B	15.6 pg/ml 15.6 pg/ml			
1C 2C	31.25 pg/ml 31.25 pg/ml			
1D 2D	62.5 pg/ml 62.5 pg/ml			
1E 2E	125 pg/ml 125 pg/ml			
1F 2F	250 pg/ml 250 pg/ml			
1G 2G	500 pg/ml 500 pg/ml			
1H 2H	1000 pg/ml 1000 pg/ml			
3A 4A	2000 pg/ml 2000 pg/ml			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	Microtiter Coated Plate (12x8 wells)
STD	Buserelin Standard, lyophilized
BIO DETN	Biotinylated Detection
STRP HRP	Streptavidin Horseradish Peroxidase
ASY DIL	Assay Diluent
1X STD DIL	(1X) Standard Diluent
1X SAMP DIL	(1X) Sample 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