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KRIBIOLISA DEGARELIX (FIRMAGON) ELISA

Cat. No: KBI5008

Ver1.0

Immunoassay for quantitative estimation of Degarelix in human 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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Introduction:

Degarelix is used for the treatment of advanced prostate cancer. Degarelix is a synthetic peptide derivative drug which binds to gonadotropin-releasing hormone (GnRH) receptors in the pituitary gland and blocks interaction with GnRH. This antagonism reduces luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which ultimately causes testosterone suppression. Reduction in testosterone is important in treating men with advanced prostate cancer. FDA approved the same as “Firmagon” in December 2008.

Intended Use:

The KRIBIOLISA Degarelix (Firmagon) ELISA is used to estimate the level of Degarelix in human serum and plasma. The kit is intended for research use only.

Principle:

The ELISA is a competitive immunoassay for the determination of Degarelix. A constant concentration of Degarelix coated on the microplate and varying concentration of standard or sample will compete for binding to gonadotropin-releasing hormone (GnRH) receptor. Detection antibody against GnRH conjugated to HRP is added to form a complex. The complex will produce a soluble colored product on substrate addition. 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 Degarelix present in standards or samples.

Materials Provided:

1. Degarelix coated microtiter plate (12 x 8 wells) - 1 no
2. GnRH Reagent - 1 vial
3. Degarelix Standards - 0, 62.5, 125, 250, 500, 1000 and 2000 ng/ml
4. Anti-GnRH:HRP Conjugate - 12 ml
5. TMB Substrate - 12 ml
6. Assay Diluent - 25 ml
7. (20X) Wash Buffer - 25 ml
8. Stop Solution - 12 ml
9. 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

Handling / Storage:

1. Reconstitute or dilute only the specific reagents mentioned in the reagent preparation section, when ready to run the assay.
2. All kit components should be stored in the refrigerator (2- 4°C) up to the kit’s expiration date.
3. Do not use kit components after the expiration date.

4. Before using, bring all components to Room Temperature (18-25ºC). Upon assay completion return all components to appropriate storage conditions.
5. ELISA plate pouches contain desiccant. Keep the plates sealed in the pouch with desiccant in the refrigerator when not in use.

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:

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Cell Culture Supernatants - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:
1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or at -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quality of our in-house data.

Reagents Preparation (all reagents should be diluted immediately prior to use):

1. Equilibrate unopened kit components to room temperature. To avoid accumulation of moisture do not open reagents and microtiter plate while they are cold.
2. To make Wash Buffer (1X); dilute 50ml of **20X Wash Buffer** in 950ml of DI water.

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. Avoid assay of Samples containing Sodium Azide (NaN3), as it could destroy the HRP activity of the conjugate resulting in under-estimation of the antibodies.
3. All Standards and Samples should be assayed at least 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 compromising 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. Dispense **100 ul** of **Standards, Samples** into each well.

2. Pipette **100 ul** of **GnRH Reagent** into each well.

3. **Incubate** at **Room Temperature** for **60 minutes**.

2. Aspirate and wash plate 4 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.

3. Add **100 ul** of **Anti-GnRH:HRP Conjugate** into each well.

4. **Incubate** at **Room Temperature** for **60 minutes**.

4. Aspirate and wash plate 4 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.

5. Dispense **100 ul** per well of **TMB Substrate**. Cover the plate with a parafilm and **incubate** at **Room Temperature** under dark for 15-30 minutes.

8. Add **100 ul** of **Stop Solution** to each microwell.

9. Measure the optical density of the wells on a plate reader at 450 nm within 10 minutes.

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 Degarelix 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 Degarelix 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 2000 ng/ml standard.

Precautions:

1. Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.
2. Substrate is light and heat sensitive hence do not expose it to direct sunlight while pipetting or incubating.
3. Samples and kit reagents after use should be disposed of observing appropriate regulations.
4. If necessary it is recommended that the results should be confirmed by an alternative method.
5. Do not dilute or adulterate test reagents or use samples not called for in the test procedure.

Assay Characteristics:

Sensitivity:
The minimum detectable dose of Degarelix is typically less than 40 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.

Specificity:
The kit is specific as it uses GNRH receptor and monoclonal antibodies to GnRH. The Degarelix standards used are calibrated against commercially sourced Firmagon™.

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

Inter-assay Precision: 3 samples with low, middle and high level human Degarelix were tested on 3 different plates, 8 replicates in each plate.
CV (%) = SD/mean x 100
Intra-Assay: CV<10%
Inter-Assay: CV<12%

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.

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 product; 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 product 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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Krishgen Biosystems, 2018

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