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User's Manual

Degarelix (Firmagon) ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

General Description

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.

Principles of Testing

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.

Reagents And 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 Required But Not Supplied

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

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 (18-25°C). 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.

Specimen Collection And Preparation

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.

Reagent Preparation

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.

Assay Procedure

1. Dispense 100 ul of Standards, Samples into each well.

Pipette 100 ul of GnRH Reagent into each well.

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. 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.
6. Add 100 ul of Stop Solution to each microwell.
7. Measure the optical density of the wells on a plate reader at 450 nm within 10 minutes.

Calculation

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.

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%

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™.

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.