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DCM009-11
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LH ELISA

for routine analysis

Direct immunoenzymatic determination of the luteinizing hormone (LH) in human serum or plasma.

IVD

LOT
See external label

2°C 8°C

Σ = 96 tests

REF DKO009

INTENDED USE

Eagle Biosciences LH ELISA Assay Kit is a solid phase enzyme immunoassay for the quantitative determination of the luteinizing hormone (LH) concentration in human serum or plasma.
LH kit is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE

Luteinizing hormone (LH) is a glycoprotein consisting of two subunits with a molecular mass of 30,000 daltons. The α-subunit is similar to other pituitary hormones [follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (HCG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule.
The α-subunit consists of 89 amino acid residues while the β-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%. The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established (1,2). In addition, the advent of in vitro fertilization (IVF) technology to overcome infertility associated problems has provided the impetus for rapid improvement in LH assay methodology from the technically demanding bioassay (3) to the procedurally simple and rapid immunoenzymometric assays.

2. PRINCIPLE OF THE TEST

In this LH ELISA Assay Kit, the LH calibrators, the patient specimens and/or the controls (containing the native antigen) are first added to streptavidin coated wells. Then monoclonal biotinylated and enzyme labeled antibodies are added and the reactants mixed: these antibodies have high affinity and specificity and are directed against distinct and different epitopes of LH. A reaction between the various LH antibodies and native LH occurs in the microwells without competition or steric hindrance forming a soluble sandwich complex.
The interaction is illustrated in the following equation:

$$\mathbf{EnzAb + AgLH + BtnAb(m) \underset{k\text{-}a}{\overset{ka}{\leftrightarrow}} EnzAb\text{ - }AgLH\text{-}BtnAb(m)}$$

BtnAb(m) = biotinylated monoclonal antibody (excess quantity)
AgLH = native antigen (variable quantity)
EnzAb = enzyme labeled antibody (excess quantity)
EnzAb-AgLH-BtnAb(m) = antigen-antibodies sandwich complex
ka = rate constant of association
k-a = rate constant of dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

EnzAb -AgLH-BtnAb(m) + StreptavidinC.W. ⇒ Immobilized complex

Streptavidin C.W. = streptavidin immobolized on well.
Immobilized complex = antibodies-antigen sandwich bound.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by a washing step. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. The activity of the enzyme present on the surface of the well quantitated by reaction with a suitable substrate to produce colour. By utilizing several different calibrators of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. LH Calibrators (6 vials, 1 mL each)

CAL0 **REF DCE002/0906-0**
CAL1 **REF DCE002/0907-0**
CAL2 **REF DCE002/0908-0** CAL3
REF DCE002/0909-0
CAL4 **REF DCE002/0910-0**
CAL5 **REF DCE002/0911-0**

2. LH Control (1 vial, 1 mL)
The Control concentration is lot-specific and it is printed on the Quality Control Report
REF DCE045/0903-0

3. Conjugate (1 vial, 12 mL)
Antibodies anti LH conjugated with Horseradish peroxidase (HRP) and anti LH biotinilated
REF DCE002/0902-0

4. Coated Microplate (1 breakable microplate)
Streptavidin adsorbed on microplate
REF DCE002/0903-0

5. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF DCE004-0

6. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15 mol/L *(avoid any skin contact)*
REF DCE005-0

7. 50X Conc. Wash Solution (1 vial, 20 mL)
NaCl 45 g/L; Tween-20 55 g/L **REF DCE006-0**

3.1. Reagents necessary not supplied

Distilled water.

3.2. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm)

Note

Store all reagents at 2÷8°C in the dark.
Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use.

4. WARNINGS

- This LH ELISA Assay Kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents of the LH ELISA Assay Kit contain small amounts of Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This LH ELISA Assay Kit allows the determination of LH from 5 mIU/mL to 200 mIU/mL.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the LH ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all LH ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange LH ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_5)

The Calibrators are ready to use, are calibrated against the international reference WHO 1st IRP 68/40 and have the following concentrations:

	C_0	C_1	C_2	C_3	C_4	C_5
mIU/mL	0	5	25	50	100	200

For sample with concentration over 200 mIU/mL dilute the sample with the Calibrator 0.

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Wash solution

Dilute the contents of each vial of the "50X Conc. Wash Solution" with distilled water to a final volume of 1000 mL prior to use. For smaller volumes respect the 1:50 dilution ratio. The diluted wash solution is stable for 30 days at 2÷8°C.

6.3. Preparation of the sample

The specimens shall be serum or plasma; the usual precautions in the collection of venipuncture samples should be observed.
For accurate comparison to established normal values, a fasting morning serum or plasma sample should be obtained.
To obtain serum samples, the blood should be collected in a venipuncture tube without additives or anti-coagulants; allow the blood to clot; centrifuge the specimen to separate the serum from the cells.
Samples may be refrigerated at 2÷8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, they may be stored at -20°C for up to 30 days. Avoid repetitive freezing and thawing.
When assayed in duplicate, 0,040 mL of the specimen is required.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C).**
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_5), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Control	Blank
Sample/ Control		**20 µL**	
Calibrator C_0-C_5	**20 µL**		
Conjugate	**100 µL**	**100 µL**	
Incubate at room temperature (22÷28°C) for 1 hour. Remove the contents from each well and wash the wells 3 times with 300 µL of diluited Wash Solution.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate at room temperature (22÷28°C) for 15 minutes in the dark.			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

7. QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

5.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C_0-C_5) and of each sample.

5.2. Calibration Curve

Plot the mean value of absorbance (Em) of the calibrators (C_0-C_4) against concentration. Draw the best-fit curve through the plotted points. (es: Four Parameter Logistic).

5.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in mIU/mL.

9. EXPECTED VALUES

Reference ranges are hereby reported:

	LH (mIU/mL)
MALE:	0.7 – 7.4
FEMALE:	
Follicular phase	0.5 – 10.5
Ovulation phase	18.4 – 61.2
Lutheal phase	0.5 – 10.5
Menopausal	8.2 – 40.8

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. LIMITATIONS OF THE PROCEDURE

LH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal.

Excessive dieting and weight loss may lead to low gonadotropin concentrations. Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to asses clinical status.

11. PERFORMANCE CHARACTERISTICS

11.1. Precision

11.1.1. Intra Assay Variation

Within run variation was determined by replicate (20x) the measurement of three different control sera in one assay. The within assay variability is ≤ 9.21%.

11.1.2 Inter Assay Variation

Between run variation was determined by replicate (10x) the measurements of three different control sera in different lots. The between assay variability is ≤ 7.91%.

11.2. Correlation

Diametra LH ELISA kit was compared to a commercially available LH kit. 36 serum samples were tested.
The regression curve is:
Diametra = 0.91*(commercial kit) + 0.05
R squared = 0.971

The new Diametra LH kit was compared to the old Diametra LH kit. 36 serum samples were tested.
The regression curve is:
(Diametra new) = 1.08*(Diametra old) - 1.22
R squared = 0.981

11.3. Sensitivity

The lowest detectable concentration of LH that can be distinguished from the Calibrator 0 is 0.22 mIU/mL.

11.4. Specificity

The cross-reactivity of the Diametra LH kit to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Luteinizing Hormone needed to produce the same absorbance.

Substance tested	Cross Reactivity
LH	100 %
HCG	0.007 %
Prolactin	N.D.
FSH	N.D.
TSH	N.D.

11.5. Accuracy

The recovery test performed on three different samples, enriched with 5.63 - 11.25 - 22.5 - 45 - 90 mIU/mL of LH, gave a average value (±SD) of 97.17% ± 4.00%.
In the diluition test three different samples were diluted 2, 4, 8 and 16 times with Calibrator 0; the average value (±SD) obtained is 99.13% ± 7.37%.

11.6. Hook Effect

Diametra LH assay shows no Hook Effect up to 400 mIU/mL.

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation