*For in vitro diagnostic use only.*

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# Luteinizing Hormone (LH) Assay Reagent Kit (CMIA)

# Package Insert

## INTENDED USE

**The Luteinizing Hormone (LH) Assay Reagent Kit (CMIA) is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of luteinizing hormone (LH) in human serum and plasma.**

## PACKING SIZE

24 Device/Kit,30Device/Kit,48 Device/Kit,60 Device/Kit.

## SUMMARY

Human luteinizing hormone (LH, lutropin) is a glycoprotein hormone with two dissimilar subunits (α and β). The α-subunit is essentially identical to the α-subunits of follicle stimulating hormone (FSH, follitropin), thyroid stimulating hormone (TSH, thyrotropin), and human chorionic gonadotropin (hCG). The β-subunit is considerably different from that of FSH and TSH. However, the β-subunits of LH and hCG are very similar.

LH, together with FSH, is secreted by the gonadotroph cells in the pituitary in response to the secretion of the gonadotropin releasing hormone (LHRH, GnRH) from the medial basal hypothalamus. Ovarian steroids, principally estrogens, modulate the secretion of LH and FSH which in turn regulate the menstrual cycle in females. When the follicle and the ovum contained within it, reach maturity, a surge of LH causes the follicle to rupture releasing the ovum. The follicular remnant is transformed into a corpus luteum, which secretes progesterone and estradiol. During the follicular and luteal phases, LH concentrations are much lower than the levels observed at the time of the LH surge. During the follicular and luteal phases, the estrogens exert a negative feedback on the release of LH. Shortly before the mid-cycle surge in LH, ovarian steroids, specifically estradiol, exert a positive feedback on the release of LH.

Determination of the concentration of LH is essential for the prediction of ovulation, in the evaluation of infertility, and the diagnosis of pituitary and gonadal disorders. Increasing concentrations of LH precede ovulation and in cases in which the period of optimal fertility needs to be defined for the timing of intercourse or artificial insemination, daily concentrations of LH are important for the prediction of ovulation. More frequent sampling is required if the precise time of follicular rupture is needed for egg aspiration for in vitro fertilization.

At menopause, or following ovariectomy in women, concentrations of estrogens decline to low levels. The lowered concentrations of estrogens result in a loss of the negative feedback on gonadotropin release. The consequence is an increase in the concentrations of LH and FSH.

The primary role of LH in the male is to stimulate the production of testosterone by the Leydig cells. LH, through the production of testosterone together with FSH, regulates spermatogenesis in the Sertoli cells of the seminiferous tubules of the testes. Testosterone exerts a negative feedback on the release of LH.

In sexually mature adults, gonadotropin deficiency is usually an early indication of the development of panhypopituitarism. Low concentrations of LH, FSH, and steroids are observed with this disorder. In contrast, gonadotropin secreting tumors of the hypothalamus and pituitary result in elevated concentrations of LH and FSH.

Gonadal failure, a cause of infertility, is indicated by elevated concentrations of LH and FSH accompanied by low concentrations of gonadal steroids. In the female, elevated concentrations of LH can indicate primary amenorrhea, menopause, premature ovarian failure, polycystic ovarian syndrome, hypergonadotropic hypogonadism, or ovulation. In the male, elevated concentrations of LH can result from primary testicular failure, seminiferous tubule dysgenesis (Klinefelter's syndrome), Sertoli cell failure, anorchia, or hypergonadotropic hypogonadism.

## PRINCIPLE OF TEST

The LH assay is a two-step immunoassay for the quantitative measurement of LH in human serum and plasma using CMIA technology, with flexible assay protocols.

In the first step, sample and anti-LH coated paramagnetic microparticles are combined. LH present in the sample binds to the anti-LH coated microparticles. After that, ALP-labeled anti-LH conjugate is added to create a reaction mixture in the second step. Following the wash cycle, substrate is added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of LH in the sample and the RLUs detected by the System optics.

## REAGENTS

The device is pre-dispensed with buffer needed for single use.
The device is constituted with Buffers described below is the main reagent

| Object | Content |
|---|---|
| Micro-particles Buffer | Anti-LH (mouse, monoclonal) coated Micro-particles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solid.<br>Preservative: ProClin-300. |
| Conjugate Buffer | Anti-LH (mouse, monoclonal) alkaline phosphatase (ALP) labeled conjugate in TRIS buffer with protein (bovine) stabilizer.<br>Preservative: ProClin-300. |
| Wash Buffer | TRIS buffer with surfactant.<br>Preservative: ProClin-300. |
| Substrate Buffer | AMPPD, Enhancer, Surfactant, ProClin-300. |

**Reagent Handing**

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

## MATERIALS PROVIDED

·LH Test Device
·Product Insert
·Calibration Solution (optional)
·Control Solution (optional)

## MATERIALS REQUIRED BUT NOT PROVIDED

·Analyzer

## STORAGE AND STABILITY

▪Store at 2-8℃ and avoid light.
▪Do not freeze.
▪Store the reagent kit upright prior to use.
▪Expiration date: up to the stated expiration date.

Note: The LH Reagent Kit must be stored at 2-8℃ in an upright position and must be used immediately after removal from 2-8℃ storage or the device was opened. Unused reagents should be put back into the kit in time.

## SPECIMEN COLLECTION AND STORAGE

**Specimen Types**

Validated specimen types to be used with this assay:

| Specimen Types | Collection Tubes |
|---|---|
| Human serum | Serum<br>Serum separator tubes |
| Human plasma | Sodium heparin<br>Lithium heparin<br>Potassium EDTA<br>Sodium EDTA |

Other anticoagulants have not been validated for use with this assay.

The instrument does not provide the capability to verily specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

**Specimen Conditions**

- Do not use specimens with the following conditions:
  heat-inactivated
  pooled
  grossly hemolyzed
  obvious microbial contamination
- For optimal results, serum and plasma specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens especial those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

**Preparation for Analysis**

- Follow the tube manufacturer's processing instructions for specimen collection tubes.
- Specimens must be mixed THOROUGHLY after thawing, by LOW speed vortex, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

**Specimen Storage**

| Specimen Type | Storage Temperature | Maximum Storage Time |
|---|---|---|
| Serum/Plasma | 2-8℃ | 6 days |

- If testing will be delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells.
- If testing will be delayed more than 6 days, specimens should be frozen at -10℃ or colder.
- Specimens stored frozen at -10℃ or colder for 3 months showed no performance difference.
- Avoid more than 3 freeze/thaw cycles.

**Specimen Shipping**

- Before shipping specimens, it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient, at 2-8℃ (wet ice), or frozen (dry ice). Do not exceed the storage time limitations listed above.

## INSTRUMENT

The LH Test Device is designed for use on the REALY Analyzer System.

## TEST PROCEDURE

**Assay Procedure**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions. Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the digit sequence of numbers. Bring the cooled reagents to approx. 20℃ and place on the reagent disk of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening of the device.

For this test device, the transfer volume of specimens, calibrators or controls into the sample hole is 70 µL. (No less than 70 µL.)

Reagent strips should be left at room temperature between 20 and 25 ℃ for more than 30 minutes before use and kept away from light.

In order to avoid the magnetic beads adsorbed on the side wall and top due to the upside down and side placement of the reagent strip during transportation, the reagent strip should be mixed by shaking and mixing before use. The reagent strip should be mixed upside down for about 30 seconds, and then the reagent strip should be mixed upward for about 30 seconds.The reagent strip was then gently shaken so that the magnetic beads fell completely to the bottom of the strip.

**Calibration**

Every Test Device has a barcode label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed before new lot of device be used.
Renewed calibration is recommended as follows:

- ▪After 90 days (when using the same reagent lot on the analyzer);
- ▪ As required: e.g. quality control findings outside the defined limits.

Note: Refer to Instruction of Analyzer for the procedure of calibration.

**Quality Control**

For quality control, please use Control of REALY or Control Universal.

In addition, other suitable control material can be used. Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

**Specimen Dilution Procedures**

Specimens with an LH concentration greater than 200mIU/mL will be flagged as ">200 mIU/mL" and may be diluted using Manual Dilution Procedure. Use the 1:2 dilution is recommended. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution.

## EXPECTED VALUES

Normal reference value:

Men: 1.7-8.6 mIU/mL

Women

Follicular phase: 2.4-12.6 mIU/mL

Ovulation phase: 14-95.6 mIU/mL

Luteal phase: 1-11.4 mIU/mL

Postmenopause: 7.7-58.5 mIU/mL

Advice each laboratory set up your own normal reference range.

## INTERPRETATION OF RESULTS

As interpret the results, the patient's overall clinical situation, including symptoms, medical history and other related data, should be referred to.

## LIMITATIONS

- If the LH results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits such as REALY LH Assay that employ mouse monoclonal antibodies.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Although the REALY LH Assay is specifically designed to minimize the effects of HAMA and heterophilic antibodies, assay results that are not consistent with other clinical observations may require additional information for diagnosis.

## PERFORMANCE CHARACTERISTICS

**Linearity**

The linearity of LH Reagent Kit was determined by use LH calibrator to prepare 6 different specimens, measuring all these specimens follow the test instruction and then do linear fitting, the results show that the linear correlation coefficient (r) was not less than 0.9900.

**Precision/Reproducibility**

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum. Repeatedly measured 20 times, calculating the coefficient of variation.

| Intra-assay Precision | | | |
|---|---|---|---|
| Control | Mean (mIU/mL) | SD | CV |
| Level 1 | 5.20 | 0.119 | 2.29% |
| Level 2 | 51.02 | 1.534 | 3.01% |
| Level 3 | 96.54 | 3.527 | 3.65% |

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 30 times, calculating the coefficient of variation.

| Inter-assay Precision | | | |
|---|---|---|---|
| Control | Mean (mIU/mL) | SD | CV |
| Level 1 | 5.11 | 0.241 | 4.72% |
| Level 2 | 49.13 | 1.978 | 4.03% |
| Level 3 | 97.78 | 5.314 | 5.43% |

**Analytical Sensitivity**

The analytical sensitivity is defined as the concentration of LH equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The analytical sensitivity is typically less than 0.2 mIU/mL.

**Analytical Specificity**

The specificity of the LH assay system was assessed by measuring the apparent response of the assay to various potentially cross-reactive analytes.

| Compound | Concentration | Cross-reactivity |
|---|---|---|
| FSH | 200 mIU/mL | 0.05% |
| TSH | 200 μIU/mL | 0.01% |
| hCG | 1000 mIU/mL | 0.01% |

**Interference**

The following compounds in both low-level specimen and high-level specimen show no cross-reactivity when tested with the LH Assay Reagent Kit at a concentration below:

| Compound | Concentration |
|---|---|
| Hemoglobin | 500 mg/dL |
| Bilirubin | 30 mg/dL |
| Triglycerides | 1000 mg/dL |

**Method Comparison**

The comparison between the LH Assay Reagent Kit (y) and a commercially available LH test kit (x), using clinical samples gave the following correlations (mIU/mL):

Linear regression

y=1.0411x – 0.2780

r=0.9873

Number of samples measured: 108

The sample concentrations were between about 1.0 – 200.0 mIU/mL.

## WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use.
- Do not use expired or clearly damaged kits.
- Operating according to the steps described, can make the risk of daily handling patients` samples and blood products into a minimum, however, no matter what the source of the products, handling mode or the previous proof, these potentially infectious substances were used shall be in accordance with the unified considerations and Good Laboratory Practice (GLP).
- Proper disinfectant should be used to eliminate pollution.
- Follow local rules and regulations to keep and dispose of these items and containers for these items.
- The ProClin-300 is a potential skin sensitizer. Avoid dumping or splashing this reagent on your skin and clothing. In case of contact with this reagent, wash thoroughly with soap and water.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- The reagents should be kept away from light, and unused reagents should be put back into the kit in time and be careful to avoid light.

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## SYMBOLS

| Symbol | Meaning | Symbol | Meaning |
|---|---|---|---|
| IVD | In vitro diagnostic medical device | | Storage temperature limit |
| | Manufacturer | EC REP | Authorized representative in the European Community /European Union |
| | Date of Manufacture | | Use-by date |
| | Do not re-use | | Consult instructions for use or consult electronic instructions for use |
| LOT | Batch code | | Do not use if package is damaged and consult instructions for use |
| REF | Catalogue number | Σ | Contains sufficient for <n> tests |


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