

**PRODUCT PROFILE AND INSTRUCTIONS****INTENDED USE**

The Primate LH ELISA test is an immunoassay designed for the quantitative determination of luteinizing hormone (LH) in serum/plasma samples of Primates and related species. The test is intended for professional use as a research tool in monitoring the physiological/pathological conditions related to circulating LH and fertility.

TEST PRINCIPLE

The LH ELISA Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a MONOCLONAL anti-LH antibody for solid phase (microtiter wells) immobilization and mouse anti-alpha-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 2 hours incubation, the wells are washed with wash buffer to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the absorbency is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is directly related to the amount of unlabeled LH in the sample. By reference to a series of LH standards assayed in the same way, the concentration of LH in the unknown sample is quantified.

MATERIALS PROVIDED

1. Antibody-coated microtiter wells, 96-well plate
2. Reference Standard/Ready to use/0.5mL/Vial
(0, 1, 2.5, 5, 10, 25ng/mL, store frozen -20°C)
3. Enzyme Conjugate Reagent, 12 mL
4. TMB Color Reagent (ready to use) 12 mL
5. 20X Wash buffer, 20 mL
6. Stop solution (2N HCl), 6mL
7. Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Distilled water
4. Glass tubes or flasks to prepare TMB Solution
5. Vortex mixer or equivalent
6. Absorbent paper or paper towel
7. Graph paper
8. Microtiter plate read

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum or plasma samples only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Unopened test kits should be stored at 4-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. To prepare the wash buffer add one part of the reagent buffer to 19 parts of distilled water. Prepare desired amount and excess solution can be stored (refrigerated) and is stable for one week.
3. Ready to use Standards should be kept frozen, if not used immediately.

ASSAY PROCEDURE

One must follow accurately these steps to ensure correct results. Use clean pipettes and sterile, disposable tips:

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 ul of standards, specimens, and controls into appropriate wells. If you choose to use lower volume you may need to work out longer incubation time. One must establish incubation conditions that suite to your lab conditions before analyzing lots of samples.
3. Dispense 100ul of Enzyme Conjugate into each well. Mix for 30 seconds. It is very important to have complete mixing at this step.
4. Incubate at 37°C for 2 hours.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times with wash buffer.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100ul of TMB solution into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes, in the dark.
10. Stop reaction by adding 50 ul of 2N HCl to each well.
11. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
12. Read optical density at 450nm with a microtiter well reader.

Important note: The wash steps are very critical. Insufficient washing will result in poor precision and falsely elevated absorbency readings.



CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbency values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of LH in ng/ml from the standard curve.

Limitations & Warranty

The present ELISA is designed for helping the scientist to analyze test samples only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the ELISA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

EXPECTED VALUES AND SENSITIVITY

Each laboratory must establish its own normal ranges based on your laboratory animals. The minimal detectable concentration of Rat Luteinizing hormone by this assay is estimated to be about 0.5ng/ml.

REFERENCES

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PRIMATE LH

PRIMATE LUTEINIZING HORMONE (LH) ELISA TEST

QUALITY CONTROL DATA

Quality Control Data:

It is highly recommended that each laboratory must establish their own internal controls and normal reference values for desired age, sex and physiological parameters.

A typical standard curve (illustration only) for a primate LH is given below:

Standard ng/mL	OD at 450nm
0	0.05
1.0	0.33
2.5	0.61
5.0	0.92
10	1.55
25	2.84

ELISA Performance Characters

Precision: Inter and Intra assay variation (CV) was determined from three different pooled serum samples in three different experiments.

Inter-assay variation	Set1: CV= 6.1% (N=10)	Set2: CV= 5.9 % (N=10)	Set3: CV= 6.1 % (N=10)
Intra-assay variation	Set1: CV= 5.8% (N=10)	Set2: CV= 7.4 % (N=10)	Set3: CV= 6.8 % (N=10)

Sensitivity: The lowest level detectable in this assay is 0.5 ng/mL of serum or plasma

Specificity: The primate LH ELISA system utilizes monoclonal antibody and high affinity polyclonal antibody to LH. The cross reactivity to other pituitary gonadotropins (TSH, FSH, GH and Prl) is not detectable under the sensitivity of the assay system.

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