Human LH ELISA Kit

to determine Human LH in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LT2096ETKKBA

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INTENDED USE

This kit is used to determine Human LH in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.02

ASSAY SUMMARY

Luteinizing hormone (LH, also known as lutropin and sometimes lutrophin) is a hormone produced by gonadotropic cells in the anterior pituitary gland. LH is an important hormone of the reproductive system, which has found application in diagnosis and therapeutic medicine. It plays a vital role in the development and functioning of the reproductive system. Determination of LH concentration is important for detection of dysfunction of the pituitary variance axis, diagnosis of reproductive disorders, monitoring of antifertility programmes and in therapeutic preparations. Human Luteinizing hormone (LH) ELISA Kit employs a two-site sandwich ELISA to quantitate LH in samples. An antibody specific for LH has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LH present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated Mouse LH detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of LH bound in the initial step. The color development is stopped and the intensity of the color is measured.

Detection range

2 IU/L-75 IU/L

Sensitivity

The minimum detectable dose (MDD) of Human LH is typically less than 0.5 IU/L. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Recovery

The recovery of Human LH spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.

The recovery ranged from 90% to 110% with an overall mean recovery of 100%.

Linearity

To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human LH were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Specificity

Human LH ELISA Kit has high sensitivity and excellent specificity for detection of Human LH. No significant cross-reactivity or interference between Human LH and analogues was observed.

Precision

Intra-assay Precision (Precision within an assay)

Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)<15%.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%)<15%.

GENERAL GUIDELINES

• Do not mix or substitute reagents with those from other lots or sources.

• It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.

• If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.

• Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

• This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

• Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided.

• Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips

4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

PRODUCT INFORMATION

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

Kit components	Storage conditions
Human LH microplate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human LH standard	May be stored for up to 6 months at 2-8 °C.
Human LH detect antibody	May be stored for up to 1 year at 2-8 °C.
HRP substrate A	
HRP substrate B	
Stop solution	
Wash buffer (20×)	

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw

cycles.

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 1000×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 1000×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.

Note: Samples should be centrifuged adequately and no hemolysis or granule was allowed.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Human LH standard - Briefly spin a vial of lyophilized standard. Add 700 µl of Deionized or distilled water into the lyophilized standard vial. Dissolve the powder thoroughly by gentle mixing.

Wash buffer - Dilute with Distilled or deionized water 1:20.

Sample dilution - If your samples need to be diluted, Deionized or distilled water is used for dilution of serum/plasma samples.

ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.

2. Add Standard/Sample: Set Standard wells, testing sample wells. Add standard 50 µl to standard well. Add testing sample 50 µl to testing sample wells.

3. Add 50 µl of Human LH detect antibody to each well, cover with a plate cover and incubate for 60 minutes at 37 °C.

4. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash buffer (250 µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add HRP substrate A 50 µl and HRP substrate B 50 µl to each well. Gently mix and incubate for 15 minutes at 37 °C. Protect from light.

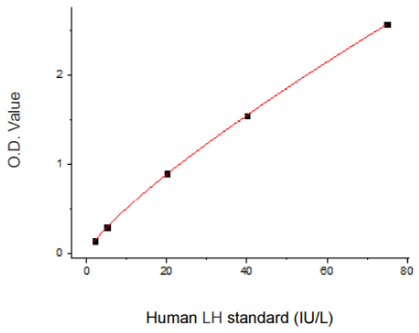
6. Add 50 µl Stop Solution to each well.

7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

CALCULATION

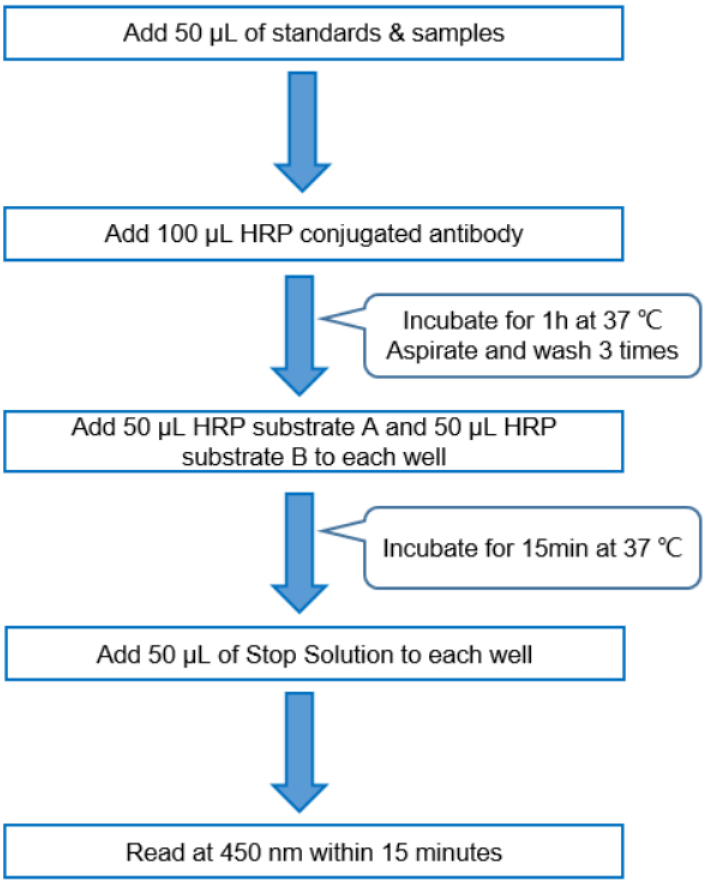
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Human LH concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA



Standard LH (IU/L)	Optical Density (450 nm)	Average	Corrected
0	0.008	0.008	—
	0.008		
2	0.140	0.144	0.136
	0.148		
5	0.296	0.2985	0.291
	0.301		
20	0.867	0.9025	0.895
	0.938		
40	1.449	1.5505	1.543
	1.652		
75	2.487	2.583	2.575
	2.679		

SUMMARY PROTOCOL



GENERAL PRECAUTIONS

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. When mixing or reconstituting protein solutions, always avoid foaming.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
7. Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

9. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).