ab178658 – Luteinizing hormone (LH) Human ELISA Kit

Instructions for Use

An immunoenzymatic assay for the quantitative measurement of Luteinizing hormone (LH) in Human serum.

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

Version 3a Last Updated 29 August 2023

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1. BACKGROUND

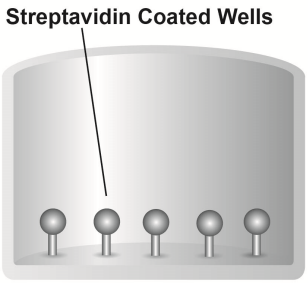
Abcam's Luteinizing hormone (LH) Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of LH in serum.

A 96-well plate has been precoated with Streptavidin. Samples, standards and the LH-HRP and Biotin conjugate are added to the wells. Biotinylated monoclonal 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. Reaction between the various LH antibodies and native LH occurs in the microwells without competition or steric hindrance forming a soluble sandwich complex. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of Luteinizing hormone in the sample and the intensity is measured at 450 nm.

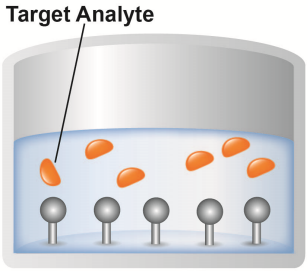
Luteinizing hormone is a glycoprotein consisting of two subunits with a molecular mass of 30,000 daltons. The α-subunit is similar to other pituitary hormones [follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (HCG)] while the β-subunit is unique. The β-subunit 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 in ascertaining the homeostasis of fertility regulation via the hypothalamic -pituitary - gonadal axis has been well established. In addition, the advent of in vitro fertilization (IVF) technology to overcome infertility associated problems has provided the impetus for rapid improvement in Luteinizing hormone assay methodology from the technically demanding bioassay to the procedurally simple and rapid immunoenzymometric assays.

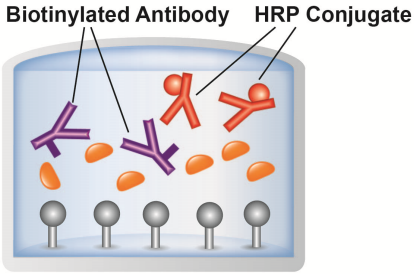
2. ASSAY SUMMARY



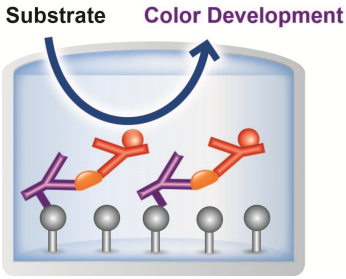
Prepare all reagents, samples and standards as instructed.



Add standard or sample to each well used. Incubate at room temperature



Add prepared Conjugation Solution to appropriate wells. Incubate at room temperature.



After washing, add TMB Substrate Solution to each well. Incubate at room temperature. Add Stop Solution. Read at 450 nm.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Luteinizing hormone Coated Microplate (12 x 8 wells)	96 Wells	2-8ºC
Stop Solution	15 mL	2-8ºC
Luteinizing hormone HRP and Biotin Conjugate	12 mL	2-8ºC
TMB Substrate Solution	15 mL	2-8ºC
50X Washing Solution	20 mL	2-8ºC
Luteinizing hormone Control	1 mL	2-8ºC
Luteinizing hormone Standard 0 – 0 mU/mL	1 mL	2-8ºC
Luteinizing hormone Standard 1 – 5 mU/mL	1 mL	2-8ºC
Luteinizing hormone Standard 2 – 25 mU/mL	1 mL	2-8ºC
Luteinizing hormone Standard 3 – 50 mU/mL	1 mL	2-8ºC
Luteinizing hormone Standard 4–100 mU/mL	1 mL	2-8ºC
Luteinizing hormone Standard 5– 200 mU/mL	1 mL	2-8ºC
Strip Holder	1 unit	2-8ºC
Cover Foil	1 unit	2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- 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
- 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
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use.

9.1 **1X Washing Solution**

Prepare 1X Washing Solution by diluting 50X Washing Solution with deionized water. To make 500 mL 1X Washing Solution combine 10 mL 50X Washing Solution with 490 mL deionized water. Mix thoroughly and gently.

- All other solutions are supplied ready to use

10. SAMPLE COLLECTION AND STORAGE

- Use Human serum samples with this assay. If the assay is performed within 5 days of sample collection, the specimen should be kept at 2-8°C; otherwise it should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing.
- Samples with concentration over 200 mU/mL should be diluted with standard 0.

Avoid repeated freezing and thawing.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates).

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

12.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

12.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

12.3. Add 20 µL standards, control and samples into their respective wells. Add 100 µL Luteinizing hormone HRP and Biotin Conjugate to each well. Leave a blank well for substrate blank.

12.4. Cover wells with the foil supplied in the kit and incubate for 1 hour at room temperature.

12.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 seconds. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid

Note: Complete removal of liquid at each step is essential for good assay performance.

12.6. Add 100 µL TMB Substrate Solution into all wells.

12.7. Incubate for exactly 15 minutes at room temperature in the dark.

12.8. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

12.9. Measure the absorbance of the sample at 450 nm within 30 minutes of addition of the Stop Solution.

13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

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

OD COVERSION - The optical densities (O.D.s) of some calibrators and samples may be higher than 2.0, in such a case, they could be out of the measurement range of the microplate reader. It is therefore necessary, for O.D.s higher than 2.0, to perform a reading at 405 nm (=wavelength of peak shoulder) in addition to 450 nm (peak wavelength) and 620 nm (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm.

- Read again the plate at 405 nm and 620 nm.

- Find out the wells whose ODs at 450 nm are higher than 2.0

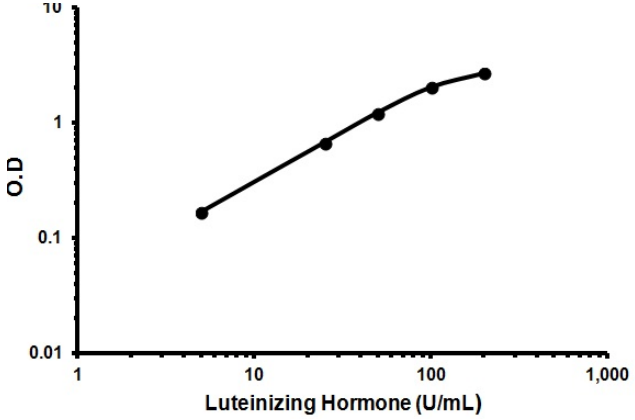
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where OD 450/OD 405 = 3.0), that is: OD 450 nm = OD 405 nm x 3.0.

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for his own reader.

VALIDATION - The OD of standard 5 should be ≥ 1.3.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



15. TYPICAL SAMPLE VALUES

REFERENCE VALUES –

The serum Luteinizing hormone values are shown in the table below:

	mU/mL
Males	0.7 – 7.4
Females follicular phase	0.5 – 10.5
Females Ovulation phase	18.4 – 61.2
Females Lutheal phase	0.5 – 10.5
Females Menopause	8.2 – 40.8

PRECISION –

	Intra-Assay	**Inter-Assay**
n=	20	15
%CV	≤ 9.21	≤ 7.91

ACCURACY –

The recovery test performed on three different samples, enriched with 5.63 - 11.25 - 22.5 - 45 - 90 mU/mL of Luteinizing hormone, gave an average value (±SD) of 97.17% ± 4.00%.

In the dilution test three different samples were diluted 2, 4, 8 and 16 times with Standard 0; the average value (±SD) obtained is 99.13% ± 7.37%.

SENSITIVITY –

The minimal detectable concentration of Luteinizing hormone by this assay is estimated to be 0.22 mU/mL.

CORRELATION –

Abcam's Luteinizing hormone (LH) Human ELISA Kit was compared to a commercially available Luteinizing hormone kit. 36 serum samples were tested.

The regression curve is:

Abcam = 0.91*(commercial kit) + 0.05

R^2= 0.971

HOOK EFFECT –

Abcam's Luteinizing hormone (LH) Human ELISA Kit shows no Hook Effect up to 400 mU/mL.

16. ASSAY SPECIFICITY

The cross-reactivity of the Abcam's Luteinizing hormone (LH) Human ELISA 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	Cross reactivity
LH	100 %
b-HCG	0.007 %
HCG	None detected
FSH	None detected
TSH	None detected

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding stop solution	Read plate immediately after adding stop solution
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

18. NOTES

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.co.jp/contactus (Japan)

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