Luteinizing Hormone (human) ELISA Kit

Item No. 500720

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
400720	LH Standard 1 (0 mIU/ml)	1 vial/1 ml
400721	LH Standard 2 (5 mIU/ml)	1 vial/1 ml
400722	LH Standard 3 (25 mIU/ml)	1 vial/1 ml
400723	LH Standard 4 (50 mIU/ml)	1 vial/1 ml
400724	LH Standard 5 (100 mIU/ml)	1 vial/1 ml
400725	LH Standard 6 (200 mIU/ml)	1 vial/1 ml
400726	Streptavidin Precoated Plate	1 plate
400727	Anti-LH-HRP + Anti-LH-Biotin Conjugate	1 vial/12 ml
400728	TMB Substrate Solution	1 vial/15 ml
400729	Stop Solution	1 vial/15 ml
400825	Wash Solution (50X)	1 vial/20 ml
400826	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Luteinizing Hormone (human) ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone:	888-526-5351 (USA and Canada only) or 734-975-3888
Fax:	734-971-3641
Email:	techserv@caymanchem.com
Hours:	M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. Materials used for **Sample Preparation** (see page 8).

INTRODUCTION

Background

Luteinizing hormone (LH) is a glycoprotein produced by the pituitary gland and consists of two subunits with a total molecular mass of approximately 30 kDa. The α-subunit is 92 amino acids long and is identical to the α-subunit of other pituitary hormones, such follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and chorionic gonadotropin (hCG). The β-subunit however, is unique to LH. It consists of 121 amino acids and confers the specific biological activity to the molecule. The carbohydrate content is between 15% and 30%. LH stimulates ovulation and the development of the corpus luteum, and maintains the function of the corpus luteum during the first two weeks of pregnancy. In males, LH stimulates the production of testosterone by the testis. It has been well established that LH is a useful clinical biomarker in determining the homeostasis of fertility regulation *via* the hypothalamic-pituitary-gonadal axis.[1,2]

About This Assay

Cayman's LH (human) ELISA Kit is an immunometric (*i.e.*, sandwich) ELISA which can be used to measure luteinizing hormone within the range of 0.5-200 mIU/ml. This assay offers specific and sensitive analysis of LH in human serum and has not been validated for other types of samples.

Principle of the Assay

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied with the kit has been coated with streptavidin. Samples and/or standards, biotinylated capture antibody and an HRP-labeled detection antibody (Anti-LH-HRP) are incubated in the wells. The biotinylated-capture antibody will bind both the streptavidin on the plate and any LH introduced into the well, whereas the detection antibody will bind a different epitope on the LH molecule. The entire complex is immobilized onto the wells by the streptavidin-biotinylated antibody interaction. After washing away excess, unbound reagents, the concentration of LH is determined by measuring the enzymatic activity of HRP by adding the substrate tetramethylbenzidine (TMB). After a sufficient period of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of this color is directly proportional to the amount of bound anti-LH-HRP, which in turn is proportional to the amount of LH.

$$\text{Absorbance} \propto [\text{Anti-LH HRP}] \propto [\text{LH}]$$

A schematic of this process is shown below in Figure 1.

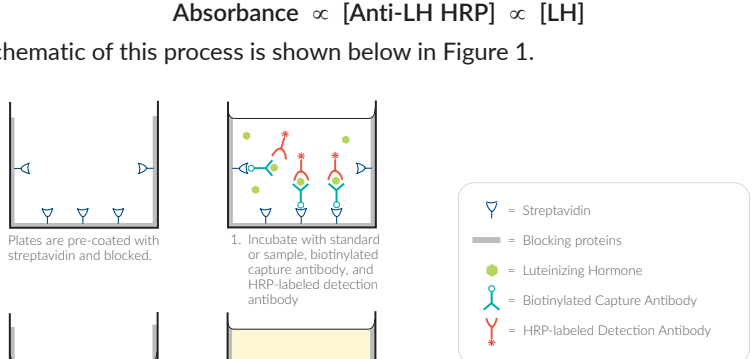


Figure 1. Schematic of the ELISA

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C.

Wash Buffer Preparation

20 ml vial Wash Solution (50X; Item No. 400825): Dilute to a total volume of 1,000 ml with distilled or deionized water.

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:50.

Sample Preparation

Human serum can be used directly in the assay.

To prepare serum samples for use in the ELISA, collect blood by venipuncture into tubes without additives or anti-coagulants and allow the blood to clot. Centrifuge the clotted blood at 1,000-2,000 x g for 15 minutes and carefully transfer the samples to clean tubes avoiding any lipid or cell debris in the tubes. For accurate comparison to established normal values, the serum sample should be collected after overnight fasting.

NOTE: Do not use heavily hemolyzed or highly lipemic samples. Store samples refrigerated (2°C-8°C) for a maximum of five days. If samples cannot be assayed within this time, store them at -20°C for up to 30 days. Avoid repetitive freeze-thaw cycles.

When assayed in dulpicate, 40 µl is required. If the concentration of LH in the sample is greater than 200 mIU/ml, dilute an aliquot of the sample with Standard 1 (0 mIU/ml).

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

NOTE: It is very important to bring all reagents, samples, and standards to room temperature (22-28°C) before starting the assay.

LH Standard (Item Nos. 400721-400725)

Each of the six vials contains 1 ml standard solution at concentrations listed in **Materials Supplied** section (see page 3), as well as listed on each vial. The standards are ready to use. After opening, the standard solutions are stable for six months if stored at 4°C.

Anti-LH-HRP + Anti-LH-Biotin Conjugate (Item No. 400727)

This vial contains 12 ml of a ready-to-use mixture of HRP-labelled Anti-LH and biotin-labelled Anti-LH antibodies.

TMB Substrate Solution (Item No. 400728)

This vial contains 15 ml of a ready-to-use TMB/hydrogen peroxide substrate solution. When stored in the dark at 4°C, the solution is stable for up to six months after opening. The solution should be colorless or have a slight blue tinge. If it is blue, it may have become contaminated and should not be used.

Stop Solution (Item No. 400729)

This vial contains 15 ml 0.15 M sulfuric acid and is ready to use.

Plate Set Up

The 96-well plate included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all of the strips at once, plate the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two blanks (Blk) and a six point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 2, see below. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest you record the contents of each well on the template sheet provided (see page 18).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	2	2	10	10	18	18	26	26	34	34
B	S2	S2	3	3	11	11	19	19	27	27	35	35
C	S3	S3	4	4	12	12	20	20	28	28	36	36
D	S4	S4	5	5	13	13	21	21	29	29	37	37
E	S5	S5	6	6	14	14	22	22	30	30	38	38
F	S6	S6	7	7	15	15	23	23	31	31	39	39
G	Blk	Blk	8	8	16	16	24	24	32	32	40	40
H	1	1	9	9	17	17	25	25	33	33	41	41

Blk - Blank
S1-S6 - Standards 1-6
1-41 - Samples

Figure 2. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

NOTE: Perform all assay steps in the order given and without appreciable delays between steps.Pipetting samples should not extend beyond ten minutes to avoid assay drift. TMB Substrate Solution and Stop Solution should be added in the same sequence.

Addition of the Reagents

1. **Luteinizing Hormone Standards**

   Add 20 µl of each standard to appropriate wells.

2. **Samples**

   Add 20 µl of sample to appropriate wells.

3. **Anti-LH Conjugate**

   Add 100 µl conjugate to each well, except the Blk wells.

Incubation of the Plate

Cover the plate with the plastic film and incubate at room temperature (22°C-28°C) for one hour.

Development of the Plate

1. Empty the wells and wash twice with diluted Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of TMB Substrate Solution to each well of the plate, including the Blk wells.
3. **Incubate for exactly 15 minutes** at room temperature in the dark.
4. DO NOT WASH THE PLATE OR EMPTY THE WELLS. Add 100 µl Stop Solution to all wells and in the same order and same rate as the addition of TMB Substrate in Step 2.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Read the plate at a wavelength of 450 nm.
3. The optical density (O.D.) of standard 6 should be ≥1.3.

ANALYSIS

Calculations

Standard Curve & Determination of Sample Concentration

Average the absorbance values of the Blk wells and subtract this value from the absorbance readings of each standard and sample well.

Using computer data reduction software, plot O.D. *versus* concentration for standards (S1-S6) and fit the data with a 4-parameter logistic equation, or alternatively, a smoothed cubic spline. Interpolate the concentration of your samples from the standard curve and be sure to correct for any dilution of the sample prior to addition to the well of the plate.

Reference Values

The following are expected ranges of LH in human serum:

Males:	0.7-7.4 mIU/ml
Females:	
Follicular Phase	0.5-10.5 mIU/ml
Ovulation Phase	18.4-61.2 mIU/ml
Lutheal Phase	0.5-10.5 mIU/ml
Menopause	8.2-40.8 mIU/ml

Performance Characteristics

Sensitivity

The minimal detectable concentration of LH by this assay is estimated to be 0.5 mIU/ml.

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

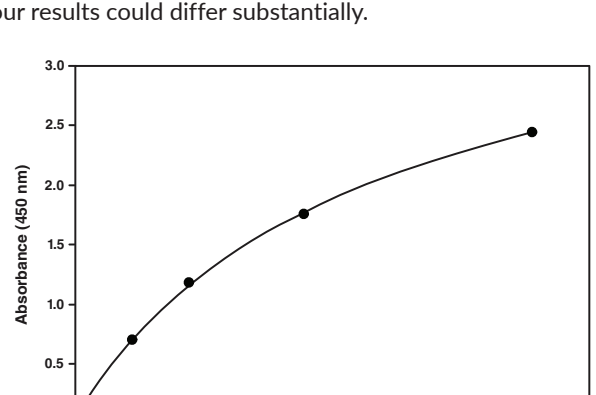


Figure 3. Typical standard curve

Precision

When a series of twenty different LH measurements of three different control sera were assayed in one assay, the intra-assay variability was ≤9.21%. When a series of LH measurements of three different control sera were assayed in 15 different assays, the inter-assay variability was ≤7.91%.

Cross Reactivity:

The cross reactivity of this kit to selected substances was evaluated by adding the potentially cross reacting substance to a serum matrix at various concentrations. The cross reactivity was calculated by deriving a ratio between the dose of test compound to dose of LH needed to produce the same absorbance.

Substance	Cross Reactivity Ratio	Concentration
Lutropin (LH)	1.0000	-
β-LH subunit	<0.0001	1,000 ng/ml
Follitropin (FSH) I	<0.0001	1,000 ng/ml
Chorionic gonadotropin (CG)	<0.0001	1,000 ng/ml
Thyrotropin (TSH)	<0.0001	1,000 ng/ml

Table 3. Cross Reactivity of the LH Assay

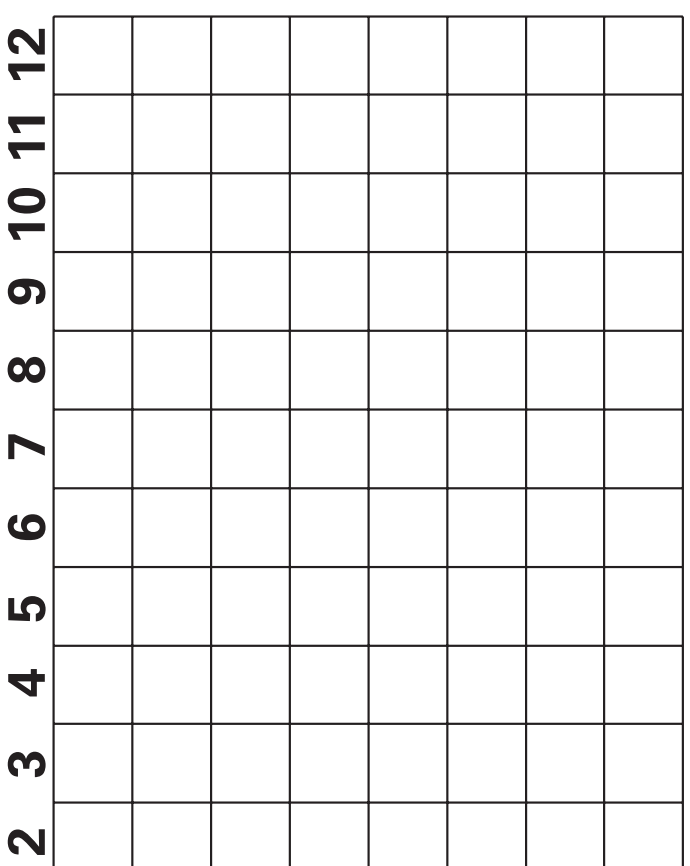
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal	A. Omission of key reagent B. Washes too stringent C. Incubation times inadequate D. Plate reader settings not optimal E. Incorrect assay temperature	A. Check that all reagents have been added in the correct order B. Use an automated plate washer if possible C. Use recommended incubation times D. Verify the wavelength and/or filter settings in the plate reader E. Use recommended incubation temperature; bring substrates to room temperature before use
High background	Inadequate washing	Ensure all wells are filled with Wash Buffer and are aspirated completely
Poor standard curve	A. Wells not completely aspirated B. Reagents poorly mixed C. Technique problem	A. Completely aspirate wells between steps B. Be sure that reagents are thoroughly mixed C. Proper mixing of reagents and wash steps are critical

References

1. Kosasa, T.S. Measurement of human chorionic gonadotropin. *J. Reprod. Med.* **26**, 201-206 (1981).
2. Danzer, H., Braunstein, G.D., Rasor, J., *et al.* Maternal serum human chorionic gonadotropin concentrations and fetal sex prediction. *Fert. Steril.* **34(4)**, 336-340 (1980).
3. Braunstein, G.D., Rasor, J., Adler, D., *et al.* Serum human chorionic gonadotropin levels throughout normal pregnancy. *Am. J. Obstet. Gynecol.* **126(6)**, 678-681 (1976).
4. Goldstein, D.P. and Kosasa, T.S. The subunit radioimmunoassay for human chorionic gonadotropin - clinical applications, *in* Progress in gynecology. Taymore, M.L. and Green, T.C., editors, 145-184 (1975).
5. Batzer, F.R. Hormonal evaluation of early pregnancy. *Fert. Steril.* **34(1)**, 1-13 (1980).
6. Braunstein, G.D., Karow, W., Gentry, W.C., *et al.* First-trimester chorionic gonadotropin measurements as an aid in the diagnosis of early pregnancy disorders. *Am. J. Obstet. Gynecol.* **131(1)**, 25-32 (1978).1
7. Lenton, E.A., Neal, L.M., and Sulaiman, R. Plasma concentrations of human chorionic gonadotropin from the time of implantation until the second week of pregnancy. *Fert. Steril.* **37(6)**, 773-778 (1982).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.