

Product Information

Luteinizing Hormone (LH) ELISA

Catalog Number **SE120071**

Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Luteinizing hormone (LH) is produced in both men and women from the anterior pituitary gland in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), which is released by the hypothalamus. LH, also called interstitial cell-stimulating hormone (ICSH) in men, is a glycoprotein with a molecular mass of ~30 kDa. It is composed of two noncovalently associated dissimilar amino acid chains, alpha and beta. The alpha chain is similar to that found in human thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG). LH stimulates ovulation and ovarian steroid production in the female. In the male, LH controls Leydig cell secretion of testosterone. LH is elevated in the luteal phase of menstrual cycle, primary hypogonadism, gonadotropin-secreting pituitary tumors, and menopause. LH is decreased in hypothalamic Gn-RH deficiency, pituitary LH deficiency, and ectopic steroid production.

The Luteinizing Hormone (LH) ELISA Kit is intended for the quantitative measurement of LH in human serum. The LH ELISA kit is a solid phase direct sandwich method. The samples and diluted anti-LH-HRP conjugate are added to the wells coated with Mab to the LH beta subunit. LH in the serum binds to anti-LH Mab on the well and the anti-LH second antibody then binds to LH. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of LH in the samples. A standard curve is prepared relating color intensity to the concentration of the LH.

Components

Material Provided	96 Tests
Microwells coated with LH MAb	12 x 8 x 1
LH Standard: 6 vials (ready to use)	0.5 ml
LH Enzyme Conjugate: 1 bottle (ready to use)	12 ml
TMB Substrate: 1 bottle (ready to use)	12 ml
Stop Solution: 1 bottle (ready to use)	12 ml
Wash concentrate 20x: 1 bottle	25 ml

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at 2–8 °C for 2 days. If storage time exceeds 2 days, store frozen at –20 °C for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Preparation of non-zero standards/calibrators

For each of the non-zero standards/calibrators (Calibrators B through F), reconstitute each vial with 2 ml of distilled or deionized water and mix. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Use the calibrators and controls as soon as possible upon reconstitution. Freeze (-20°C) the remaining calibrators and controls as soon as possible after use. Standards and controls are stable at -20°C for 6 weeks after reconstitution with up to 3 freeze thaw cycles.

20x Wash Buffer Concentrate

Prepare 1x wash buffer by adding the contents of the bottle to 475 ml of distilled water. Store 1x wash buffer at room temperature.

Storage/Stability

Store Kit at $2-8^{\circ}\text{C}$.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature ($18-26^{\circ}\text{C}$).

1. Place the desired number of coated strips into the holder
2. Pipette 50 μl of LH standards, control, and sera.
3. Add 100 μl of enzyme conjugate to all wells.
4. Cover the plate and incubate for 30 minutes at room temperature ($18-26^{\circ}\text{C}$).
5. Remove liquid from all wells. Wash wells three times with 300 μl of 1x wash buffer. Blot on absorbent paper towels.
6. Add 100 μl of TMB substrate to all wells.
7. Incubate for 10 minutes at room temperature.
8. Add 50 μl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Results

Calculations

1. Check LH standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for the LH standards (vertical axis) versus the LH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

Product Profile

Sensitivity

The sensitivity was determined by calculating the mean plus 2 SD of the standard zero point tested 20 times in the same run.

Serum	No. of Replicates	Mean mIU/ml	Standard Deviation	Mean + 2SD (Sensitivity)
Zero standard	20	0.09	0.015	0.12 mIU/ml

Correlation

Eighty samples, with ACTH values ranging from 1.5 to 1,045 pg/ml were assayed by the ACTH ELISA and a reference ELISA method.

Correlation	Slope	Intercept
0.98	0.9	0.37

Precision And Reproducibility

The precision (intra-assay variation) of the LH ELISA Test was calculated from 10 replicate determinations on each of the three samples.

Intra-Assay Variation

Serum	No.of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	16	47.2	2.92	6.18
2	16	16.3	1.24	7.60
3	16	1.7	0.18	10.58

The total precision (inter-assay variation) of the LH ELISA Test was calculated from data on three samples obtained in 10 different assays.

Inter-Assay Variation

Serum	No.of Replicates	Mean mIU/ml	Standard Deviation	Coefficient of Variation (%)
1	10	46.1	3.75	8.13
2	10	15.6	1.69	10.83
3	10	1.9	0.22	11.57

Recovery

Known quantities of LH were added to a serum that contained a low concentration of LH.

Expected Value (mIU/ml)	Recovered (mIU/ml)	Percentage of Recovery
8	8.2	103
16	14.4	90
32	33.5	104

References

1. Frank, J.E. et al., Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. J. Pediatr., 1996;128(4):548-54.
2. Thakur, C. et al., Total serum levels of triiodothyronine (T3) thyroxine (T4) and thyrotropine (LH) in school going children of Dibrugarh district: an endemic goitre region of Assam. Indian J. Physiol. Pharmacol., 1997;41(2):167-70.
3. Morimoto, K., and Inouye, K.A., Sensitive enzyme immunoassay of human thyroid-stimulating hormone (LH) using bispecific F(ab')₂ fragments recognizing polymerized alkaline phosphatase and LH. J. Immunol. Methods, 1997;205(1):81-90.
4. Maes, M. et al., Components of biological variation, including seasonality, in blood concentrations of LH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin. Endocrinol. (Oxf.), 1997; 46(5):587-98.

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