ELISA PRODUCT INFORMATION & MANUAL

Luteinizing Hormone

NBP2-61257

Enzyme-linked Immunosorbent Assay for quantitative detection of Human, Rat Luteinizing Hormone.

For research use only. Not for diagnostic or therapeutic procedures.

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Please read entire booklet before proceeding with the assay.

Please contact Novus Biologicals Technical Support if necessary.

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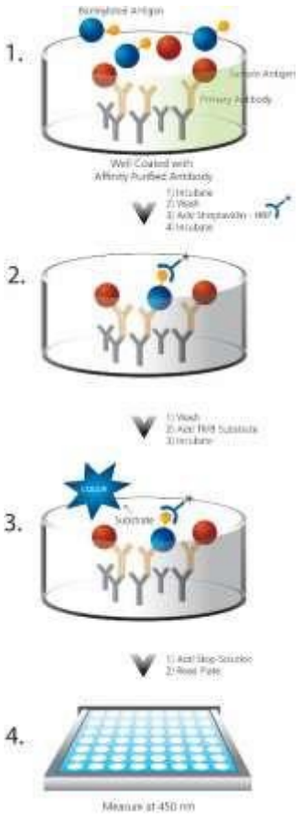
INTRODUCTION



Luteinizing hormone (LH) is 30 kDa hormone consisting of two subunits – alpha and beta. LH, FSH (follicle stimulating hormone), TSH (Thyroid Stimulating Hormone) and hCG (human chorionic gonadotropin) are glycoprotein hormones that share a common alpha subunit but have unique beta subunits.[1] The α-subunit of LH is 92 amino acids in length while the β-subunit, which is responsible for the biological activity, is 121 amino acids long.

LH is produced by the gonadotroph cells in the anterior pituitary gland. It is responsible for ovulation in women and controls testosterone synthesis in men.[2] The ovarian cycle, which highlights the synergistic nature of the glycoprotein hormones listed above, is shown below in Figure 1:

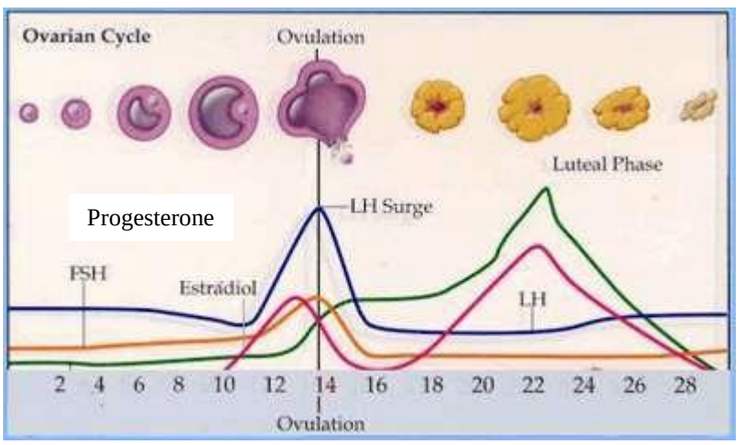


Figure 1: Ovulation Cycle and the Glycoprotein Hormones involved in Ovulation[3]

An acute rise of LH, referred to as the “LH surge”, triggers ovulation and development of the corpus luteum in females. After ovulation, LH supports the transient life span of the corpus luteum acting on the luteinized granulosa cells.[4] Cell enlargement and increased progesterone production are involved in the luteinization of the granulosa cells[5]. These cells are closely associated with the developing gamete (egg) in the ovary.

PRINCIPLE

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

1. Samples or standards are added to wells coated with a goat anti-mouse IgG antibody. A mouse monoclonal antibody to LH and a solution of biotinylated LH tracer are both added to the wells.
2. The plate is incubated. During this incubation, the antibody binds the LH in the sample or tracer in a competitive manner.
3. The plate is washed, leaving only bound LH on the plate. A solution of Horseradish Peroxidase conjugated Streptavidin (SA-HRP) is added to all wells and the plate is incubated.
4. The plate is washed to remove excess conjugate. TMB substrate is added to the wells and the plate is incubated.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is indirectly proportional to the level of LH in the sample.

MATERIALS SUPPLIED

1. **Goat anti-Mouse IgG Microtiter Plate**
   One Plate of 96 Wells
   A plate using break-apart strips coated with a goat anti-mouse IgG antibody.

2. **LH Antibody**
   5 mL
   A solution of a mouse monoclonal antibody specific to LH.

3. **Assay Buffer 13**
   50 mL
   Tris buffered saline containing proteins and detergents.

4. **LH Tracer**
   5 mL
   A solution of biotinylated LH tracer.

5. **Wash Buffer Concentrate**
   100 mL
   Tris buffered saline containing detergents.

6. **LH Standard**
   200 µL
   One vial containing 14,000 mIU/ml LH.

7. **SA-HRP Conjugate**
   20 mL
   A solution of Streptavidin-conjugated Horseradish Peroxidase.

8. **TMB Substrate**
   25 mL

   A solution of 3,3’5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide. Protect from prolonged exposure to light.

9. **Stop Solution 2**
   10 mL
   A 1N solution of hydrochloric acid in water.

10. **LH Assay Layout Sheet**
    1 each

11. **Plate Sealer**
    3 each

STORAGE

All of the components of this kit are stable at 4°C until the kit's expiration date.

OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipet for dispensing volumes between 50 µL and 200 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 450 nm.

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

SAMPLE HANDLING

This assay is suitable for measuring LH in human serum and EDTA plasma in addition to tissue culture media. Rat serum has also been validated for use in this assay. Prior to sample analysis in the assay, frozen samples should be slowly thawed at room temperature and vortexed to mix.

Neat (undiluted) samples have been validated for use in this assay (please refer to the Spike and Recovery section on page 7 for detailed data). However, due to variation in samples, a dilution *may* be required. Users must determine the optimal dilutions for their particular experiments.

In the following experiments serum, plasma and tissue culture media were utilized to determine the final recommended sample dilutions.

SAMPLE MATRIX PROPERTIES

Linearity

Neat human serum and EDTA plasma samples with biological relevant levels of LH were serially diluted in assay buffer. Additionally, neat tissue culture media was spiked with human LH and then serially diluted 1:3 in assay buffer. All samples were run in the assay and LH values were assigned using the standard curve. The percent dilutional linearity values are shown in the table below.

Dilutional linearity, %						
Dilution	**Follicular**	**Mid-Cycle**	**Luteal**	**Male Serum**	**EDTA Plasma**	**Tissue Culture Media**
Neat	131	144	87	54	65	129
1:3	77	82	58	77	79	124
1:9	100	100	100	100	100	100

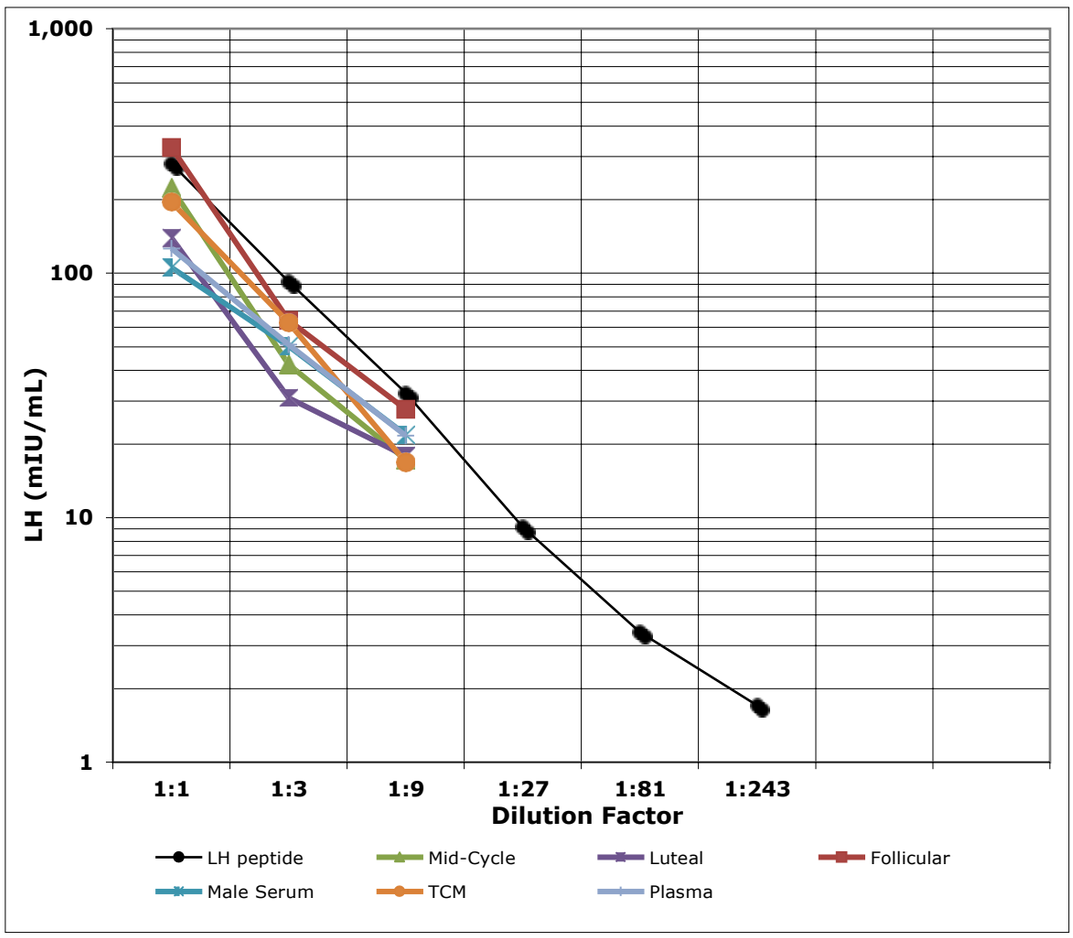
Spike and Recovery

LH was spiked at three concentrations into neat (undiluted) human serum, EDTA plasma or tissue culture media. Matrix background was subtracted and the recovery was compared to the recovery of identical LH spiked into assay buffer. The average percent recovery for each matrix at the minimum recommended dilution is indicated below.

Sample	Spike Concentration, mIU/mL	% Recovery	Minimum Recommended Dilution
Serum	200	98	Neat
	100	90	
	50	88	
EDTA Plasma	200	119	Neat
	100	131	
	50	85	
Tissue Culture Media	200	85	Neat
	100	63	
	50	76	

Parallelism

To assess parallelism, neat human serum and EDTA plasma samples were serially diluted in assay buffer. Additionally, neat tissue culture media was spiked with human LH and serially diluted in assay buffer. All of the samples were then run in the assay. The LH concentration in each sample was assigned using the standard curve. Assigned concentrations were plotted as a function of sample dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human origin.



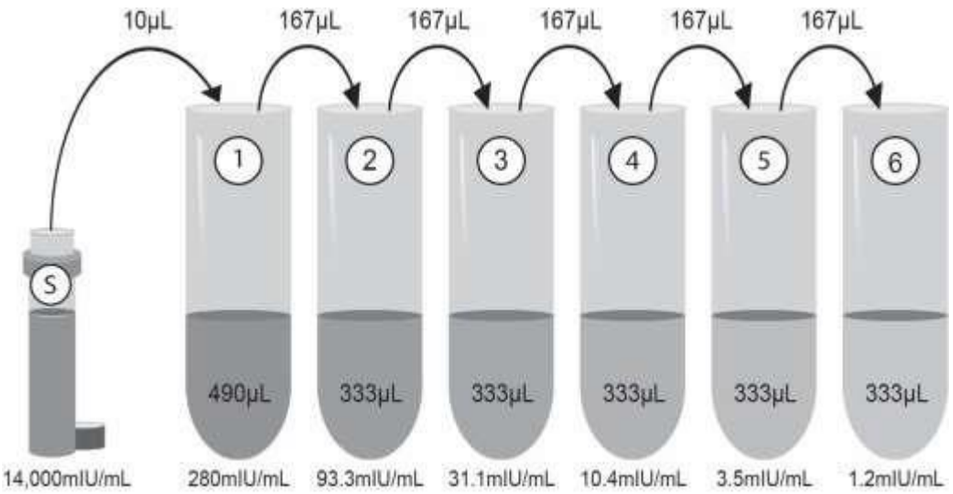
REAGENT PREPARATION

1. Wash Buffer

Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. LH Standard Curve

The LH standard stock as well as diluted standards and samples should be kept on ice and used within 60 minutes of preparation for optimal performance. Allow the LH standard to warm to room temperature.



Label six 12x75 mm polypropylene tubes #1 through #6. Pipet 490 µL of Assay Buffer 13 into tube #1. Pipet 333 µL of Assay Buffer 13 into tube #2 through tube #6. Add 10 µL of 14,000 mIU/ml LH standard stock to tube #1 and vortex. Add 167 µL of tube #1 into tube #2 and vortex. Add 167µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6.

Diluted standards should not be stored for re-use. Make new standard preparations with each use.

ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove unneeded wells and return them, with the desiccant, to the plate bag and seal. Store the unused wells at 4°C.

1. Pipet 100 µL of the assay buffer into the Bo (0 mIU/mL standard) wells and 150 µL of the same assay buffer into the NSB wells.
2. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
3. Pipet 100 µL of the Samples into the appropriate wells.
4. Add 50 µL of the LH Tracer to all wells except for the blank.
5. Add 50 µL of the LH Antibody to all wells except for the NSB and blank.
6. Seal the plate and incubate at room temperature (RT) on a plate shaker for 1 hour at ~500rpm*. **See note.**
7. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Pipet 200 µL of SA-HRP Conjugate into each well except the blank.
9. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500rpm*.
10. Wash as above (Step 7).
11. Pipet 200 µL of TMB Substrate solution into each well. Incubate for 30 minutes at RT on a plate shaker at ~500rpm*.
12. Pipet 50 µL of the stop solution into each well.
13. After zeroing the plate reader against signal amplification blank, read optical density at 450nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*** The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.**

CALCULATION OF RESULTS

The concentration of LH can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

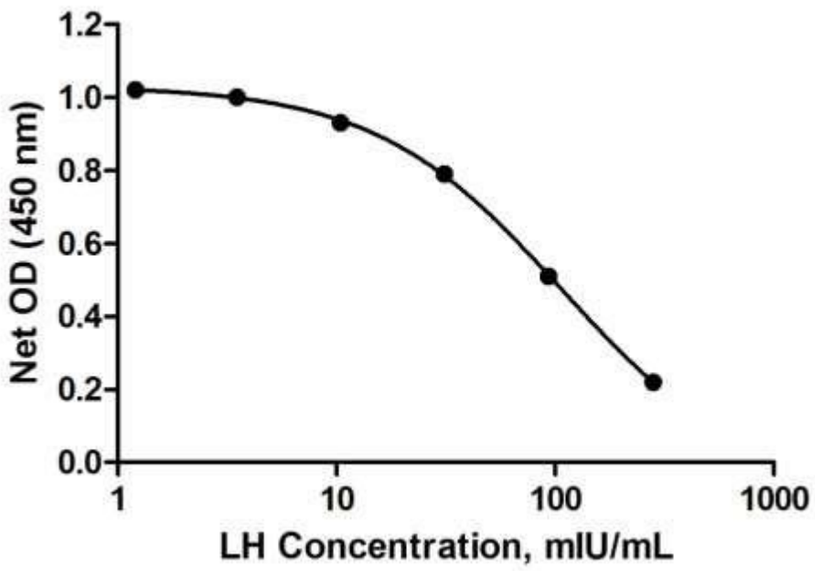
2. Using data analysis software, plot the Average Net OD for each standard versus LH concentration in each standard.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Sample	Optical Density (450nm)	LH (mIU/mL)
NSB	0.020	**n/a**
Bo	1.04	**0**
S1	0.22	**280**
S2	0.51	**93.3**
S3	0.79	**31.1**
S4	0.93	**10.4**
S5	1.00	**3.5**
S6	1.02	**1.2**

** LH conversion: 8.5x10^6 m IU/mL = 1mg/mL*



PERFORMANCE CHARACTERISTICS

Specificity

The specificity of the assay was determined by diluting the cross reactant in the kit assay buffer at a concentration of thirty times the high standard and then measuring in the assay.

Analyte	Cross Reactivity
LH	100%
FSH	≤0.004%
TSH	0.3%
hCG	≤0.004%

Sensitivity

The sensitivity or limit of detection of the assay is 5.2 mIU/mL, determined by interpolation at 2 standard deviations away from the net OD of 14 zero standard replicates. Data was used from 10 standard curves.

Intra-assay precision was determined by assaying 20 replicates of three matrix controls containing LH in a single assay.

Intra-assay precision	
mIU/mL	**%CV**
61.8	3.9
30.6	5.0
17.3	7.0

Inter-assay precision was determined by measuring matrix controls of varying LH concentrations in multiple assays over several days.

Inter-assay precision	
mIU/mL	**%CV**
80.3	15
39.2	14.9
24.2	16.7

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