



# Ultra-Sensitive Luteinizing Hormone ELISA RUO

## AL-188

### INTENDED USE

The Ultra-Sensitive Luteinizing Hormone (LH) immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Luteinizing Hormone (LH), in human serum. It is intended for *research use only* as a diagnosis tool for monitoring of various hormonal reproductive disorders.

### PRINCIPLE OF THE TEST

The US LH ELISA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and Unknown are added to LH antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated LH antibody solution. After the second incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP) solution. After the third incubation and washing step, the wells are incubated with substrate solution (TMB) followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of LH in the samples and calibrators.

### MATERIALS SUPPLIED

#### CAL-188A US LH Calibrator A

One vial, 4 mL, labeled A containing 0 mIU/mL of LH in protein based buffer and Pro-Clean 400. Store at 2-8°C until the expiration date.

#### CAL-188B - CAL-188F US LH Calibrators B thru F

Five vials, labeled B-F containing concentrations of approximately 0.1-9 mIU/mL of LH in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2-8°C until the expiration date. Reconstitute calibrator B-F with 1mL deionized water. Stabilize, mix well and use. Avoid repeated freeze thaw.

**Traceability:** *The US LH calibrators are traceable to the World Health Organization International preparation NIBSC code 81/535 version 2.0., dated 22/10/2014.*

US LH calibrators = 0.73 (LH WHO NIBSC code 81/535 version 2.0.)

#### CTR-188-I & CTR-188-II US LH Controls I & II

Two vials, labeled levels I and II containing low and high LH concentrations in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact control ranges. Store unopened at 2-8°C until the expiration date. Reconstitute calibrator B-F with 1mL deionized water. Stabilize, mix well and use. . Avoid repeated freeze thaw.

#### PLT-188 LH Coated Microtitration Strips

One strip holder, containing 12 strips and 96 microtitration wells with LH antibody immobilized to the inside wall of each well. Store at 2-8°C until

expiration date in the resealable pouch with a desiccant to protect from moisture.

#### ASB-205 AMH/MIS Assay Buffer

One bottle, 12 mL, containing a protein-based (BSA)-buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

#### BCR-188 US LH Biotin Conjugate—Ready-to-Use (RTU)

One bottle, 12 mL, containing LH Antibody-Biotin Conjugate in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

#### SAR-188 US LH Streptavidin-Enzyme Conjugate-Ready-to-Use (RTU)

One bottle, 12 mL, containing Streptavidin-Enzyme Conjugate in a protein-based buffer and a non-mercury preservative. Store undiluted at 2-8°C until expiration date.

#### TMB-100 TMB Chromogen Solution

One bottle, 12 mL, containing a solution of Tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2-8°C until expiration date.

#### STP-100 Stopping Solution

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2-30°C until expiration date.

#### WSH-100 Wash Concentrate A

One bottle, 60 mL, containing phosphate buffer saline solution with a nonionic detergent. Store at 2-30°C until expiration date. Dilute 25-fold with deionized water prior to use.

#### CRD-188 Calibration Card

One lot specific calibration card.

### MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250  $\mu$ L.
5. Vortex mixer.
6. Deionized water.

### WARNINGS AND PRECAUTIONS

#### For Research Use Only.

The following precautions should be observed:

- a) Follow good laboratory practice.
- b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

- c) Handle and dispose of all reagents and material in compliance with applicable regulations.

**WARNING: Potential Biohazardous Material**

This reagent may contain some human source material (e.g. serum) or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 5<sup>th</sup> Edition, 2007<sup>1</sup>.

**WARNING: Potential Chemical Hazard**

Some reagents in this kit contain Pro-Clean 400 and Sodium azide<sup>2</sup> as a preservative. Pro-Clean 400 and Sodium Azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS.

**SAMPLE COLLECTION AND PREPARATION**

- a) Serum is the recommended sample type.
- b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.
- c) Samples may be stored at 4°C if assayed within 24 hours; otherwise, samples must be stored at -20°C or -80°C to avoid loss of bioactivity and contamination.
- d) Avoid assaying lipemic, hemolyzed or icteric samples.
- e) Avoid repeated freezing and thawing of samples. Thaw samples no more than 3 times.
- f) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens<sup>3</sup>.

**PROCEDURAL NOTES**

1. A thorough understanding of this package insert is necessary for successful use of the US LH ELISA assay. It is the user's responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature (23 ± 2°C) before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit component and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, Sodium Azide, Hypochlorous acid and aromatic Chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the substrate solution into the wells. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

**PREPARATION OF REAGENTS**

1. **US LH Calibrators B-F and US LH Controls I & II:** Tap and reconstitute US LH Calibrator B-F and US LH Controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
2. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (23 ± 2°C) when stored in a tightly sealed bottle.
3. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

**SAMPLE PREPARATION**

Dilution of serum specimens should be performed on the same day prior to testing.

1. For each unknown serum sample, label one eppendorf vial appropriately and add 40 µL of US LH Calibrator A to each vial.
2. Add 10 µL of the serum specimens to the pre-labeled vials and vortex well.
3. The samples are now ready to be assayed.

**ASSAY PROCEDURE**

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, Controls, and Unknowns should be assayed in duplicate.

**NOTE:** All samples reading higher than the highest calibrator should be diluted in US LH Calibrator A/ Sample Diluent prior to assay (Refer sample preparation)

1. Label the microtitration strips to be used.
2. Pipette **10 µL** of the reconstituted Calibrators, Controls, and diluted unknowns to the appropriate wells
3. Add **100 µL** of the AMH/MIS Assay Buffer to each well using a repeater pipette.
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
5. Aspirate and wash each strip **5 times** with Washing Solution (**350 µL/per well**) using an automatic microplate washer.
6. Add **100 µL** of the US LH Biotin conjugate RTU solution to each well using a repeater pipette.
7. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
8. Aspirate and wash each well 5 times (350 µL per well) with the Wash Solution using an automatic microplate washer.
9. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
10. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
11. Aspirate and wash each well 5 times (350 µL per well) with the Wash Solution using an automatic microplate washer.
12. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid direct exposure to heat and sunlight.
13. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **8-12 min** at room temperature (23 ± 2°C).  
NOTE: Visually monitor the color development to optimize the incubation time.
14. Add **100 µL** of the Stopping Solution to each well using a repeater pipette.
15. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to 450 nm.

**NOTE:** Zero calibrator should be programmed as "Blank" while reading the optical density. If instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.

## RESULTS

**NOTE:** The results in this package insert were calculated by plotting the log optical density (OD) data on the y-axis and log LH concentration on X-axis using a cubic regression curve-fit. Alternatively, log vs. log quadratic regression curve-fit can be used. Other data reduction methods may give slightly different results.

- Optimum results can be obtained at incubation temperature of  $23 \pm 2^{\circ}\text{C}$ .
- Calculate the mean OD for each Calibrator, Control, or Unknown.
- Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the LH concentrations in mIU/mL along the x-axis, using a cubic regression curve-fit.
- Determine the LH concentrations of the Controls and Unknowns from the calibration curve by matching their mean OD readings with the corresponding LH concentrations.
- Any sample reading higher than the highest Calibrator should be appropriately diluted with Calibrator A/ Sample Diluent and re-assayed.
- Any sample reading lower than the analytical sensitivity should be reported as such.
- Multiply the specimen concentration obtained in the assay by a dilution factor, if required.

## LIMITATIONS

The reagents supplied in this kit are optimized to measure LH levels in human serum. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples<sup>4</sup>.

The US LH ELISA results should be interpreted with respect to the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate patient examination information.

## QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- US LH ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for LH controls are printed on the **Calibration card**.
- A full calibration curve, low and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

## REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean OD	Conc (mIU/mL)
A1, A2	A	0.026 (Blank)	0
B1, B2	B	0.042	0.097
C1, C2	C	0.125	0.31
D1, D2	D	0.46	1.2
E1, E2	E	1.39	4.2
F1, F2	F	3.02	9.9

**CAUTION:** The above data must not be employed in lieu of data obtained by the user in the laboratory.

## ANALYTICAL CHARACTERISTICS

All analytical characteristics are stated in mIU/mL.

### Analytical Sensitivity:

The analytical sensitivity in the assay as calculated by the interpolation of mean plus two standard deviation of 16 replicates of Calibrator A (0 mIU/mL) and Calibrator B (0.1 mIU/mL) is 0.006 mIU/mL.

### Imprecision:

Reproducibility of the US LH ELISA assay was determined on four QC controls (n=24). Representative data calculated are presented in the following table.

SUMMARY:		Within run			Between run		Total	
Sample ID	Runs	Mean	SD	CV	SD	CV	SD	CV
QC1	24	0.077	0.005	6.14%	0.004	5.28%	0.006	8.10%
QC2	24	0.519	0.017	3.34%	0.015	2.87%	0.023	4.40%
QC3	24	3.186	0.102	3.20%	0.084	2.64%	0.132	4.15%
QC4	24	6.190	0.208	3.36%	0.102	1.65%	0.232	3.74%

### Linearity:

Calibrator F and three serum samples containing various LH levels were diluted with calibrator A. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution factor	Expected Value in mIU/mL	Observed Value in mIU/mL	%Recovery
Cal F	NEAT VALUE	8.8	NA	NA
	2	4.4	4.21	96%
	4	2.2	2.13	97%
	8	1.1	1.07	97%
	16	0.55	0.52	95%
	32	0.28	0.28	100%
1	NEAT VALUE	8.62	NA	NA
	2	4.31	4.54	105%
	4	2.15	2.31	107%
	8	1.08	1.15	107%
	16	0.54	0.58	108%
	32	0.27	0.29	107%
2	2	7.78	NA	NA
	4	3.89	3.85	99%
	8	1.95	1.97	101%
	16	0.97	0.99	101%
	32	0.49	0.49	100%
	64	0.24	0.25	103%
3	2	5.63	NA	NA
	4	2.82	2.78	99%
	8	1.41	1.41	100%
	8	0.70	0.72	102%
	16	0.35	0.35	100%
	32	0.18	0.17	97%

### Recovery:

Known amounts of LH were added to three serum samples containing different levels of endogenous LH. The concentration of LH was determined before and after the addition of exogenous LH and the percent recovery was calculated.

Sample	Endogenous Conc. (mIU/mL)	Expected Concentration (mIU/mL)	Observed Concentration (mIU/mL)	%Recovery
1	6.42	6.61	6.25	95%
		6.77	6.61	98%
		6.95	6.51	94%
2	3.72	4.05	4.05	100%
		4.34	4.21	97%
		4.66	4.56	98%
3	3.41	3.75	3.53	94%
		4.06	3.87	96%
		4.39	4.25	97%

**Analytical Specificity:**

The monoclonal antibody pair used in the assay detects Human Luteinizing hormone and did not show any significant cross-reaction to FSH and hCG.

Intralipids	20 mg/mL	4.48	4.51	0.02
	10 mg/mL	4.71	4.67	-0.04
	5 mg/mL	4.83	4.99	0.16

**Species Immunoreactivity:**

The antibody pair used in US LH ELISA assay does not detect Rabbit, Goat, Bovine, Canine, Equine, Feline, Ovine, Porcine, Mouse, Rat, Squirrel Monkey, and Vervet Monkey samples as represented in the table below.

Sample#	Species	Type	O.D.	Conc. (mIU/mL)
1	Rabbit	Serum	-0.001	ND
2	Rabbit	Serum	-0.006	ND
3	Goat	Serum	-0.004	ND
4	Goat	Serum	0	ND
5	Bovine	Serum	0.002	<0.067
6	Bovine	Serum	0	<0.067
7	Canine	Tissue Extract	0.005	<0.067
8	Canine	Tissue Extract	0.008	<0.067
9	Canine	Serum	0.004	<0.067
10	Canine	Serum	0.014	<0.067
11	Equine	Cyst Fluid	0.004 (1:50)	<3.35
12	Equine	Serum	-0.001	ND
13	Equine	Serum	0.004	<0.067
14	Feline	Serum	0	ND
15	Feline	Serum	0.005	<0.067
16	Ovine	Serum	0.003	<0.067
17	Ovine	Serum	0.002	<0.067
18	Porcine	Serum	0	<0.067
19	Porcine	Serum	0.008	<0.067
20	Mouse	Serum	0.003 (1:10)	<0.67
21	Mouse	Serum	0.002 (1:10)	<0.67
22	Mouse	Serum	0.003 (1:10)	<0.67
23	Rat	Serum	0.004	<0.67
24	Rat	Serum	0.004	<0.67
25	Squirrel Monkey	Serum	-0.001	ND
26	Squirrel Monkey	Serum	0.004	<0.067
27	Vervet Monkey	Serum	0.004	<0.067

ND: Not detectable

**Hook Effect:**

There is no high-dose effect at LH concentration up to 109.38 mIU/mL.

**Interference:**

When potential interferents (Hemoglobin, Bilirubin, Biotin and Intralipids) were added at least at two times their physiological concentration to control sample, LH concentrations were within  $\pm 10\%$  of the control as represented in the following table.

Interferent	Interferent Dose	Sample LH (mIU/mL)	Dosed Sample LH (mIU/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	6.68	6.70	0.02
	0.5 mg/mL	7.09	7.18	0.09
	0.1 mg/mL	7.38	7.35	-0.02
Hemoglobin	1 mg/mL	4.33	4.29	-0.04
	0.5 mg/mL	4.52	4.48	-0.04
	0.1 mg/mL	4.68	4.75	0.07
Bilirubin	0.66 mg/mL	4.94	4.84	-0.10
	0.2 mg/mL	6.83	6.65	-0.18
Bilirubin	0.66 mg/mL	3.14	3.04	-0.10
	0.2 mg/mL	4.27	4.30	0.03
Biotin	1200 ng/mL	6.71	6.64	-0.06
	600 ng/mL	7.13	7.15	0.01
	200 ng/mL	7.39	7.26	-0.13
Biotin	1200 ng/mL	4.36	4.32	-0.05
	600 ng/mL	4.51	4.52	0.01
	200 ng/mL	4.74	4.57	-0.16
Intralipids	20 mg/mL	6.95	7.00	0.05
	10 mg/mL	7.19	7.25	0.06
	5 mg/mL	7.45	7.43	-0.02

**Sample Type:**

Eleven matched serum, lithium-heparin plasma and K<sub>2</sub>EDTA plasma specimens in the range of 0.5-18.0 mIU/mL were compared in LH CLIA assay (AL-288).

Passing-Bablok analysis of the results yielded the following regression:

Li-Heparin plasma = 0.006046 + 1.085 Serum (r= 0.999)

K<sub>2</sub>-EDTA plasma = 0.01816 + 1.029 Serum (r=0.998)

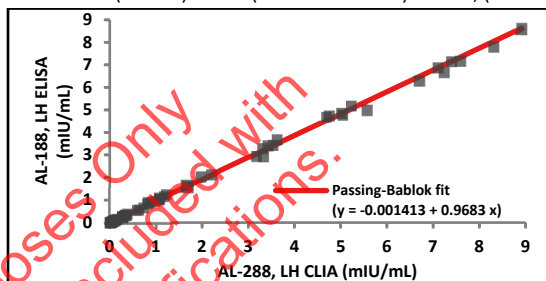
K<sub>2</sub>-EDTA plasma = -0.01265 + 1.004 Li-Heparin plasma (r=0.999)

**Method Comparison:**

The Ultra-Sensitive LH ELISA (AL-188) has been compared to AnshLite LH CLIA (AL-288) assay using 70 serum samples in the range of 0.02-9.0 mIU/mL.

Passing Bablok analysis of the results yielded the following Regression:

US LH ELISA (AL-188) = 0.97 (AnshLite LH CLIA) - 0.001, (r=0.99)

**Expected Values:**

Cycling female serum samples (day 2-4) were analyzed using Anshlabs US LH ELISA. The expected ranges were calculated between the ages of 24 and 43 years and is shown in the table below.

Females Age (years)	No of specimens (n)	Median LH conc. (mIU/mL)	LH Range (mIU/mL)
24-29	13	4.2	2.7 - 11.4
30-35	34	4.5	1.1 - 11.6
36-39	22	4.4	1.5 - 15.7
40-43	24	6.2	1.2 - 13.6

The expected ranges for LH in pediatric male samples in the age range of 3.0 – 18.0 years were calculated using 95% non-parametric estimation. A total of 368 samples in Pubic Hair Tanner stages 1 - 5 were evaluated in US LH ELISA using Analyse-It<sup>®</sup> for Microsoft Excel as seen in table below.

Pubic Hair Tanner Stage	No of specimens (n)	Median Conc. (mIU/mL)	LH (mIU/mL) 95% CI
1	183	0.07	0.014 - 1.4
2	53	0.8	0.03 - 3.3
3	32	2.3	0.6 - 5.4
4	50	2.6	0.9 - 5.8
5	50	4.2	1.5 - 8.0

The expected ranges for LH in pediatric female samples in the age range of 2.4 – 18.0 years were calculated using 95% non-parametric estimation. A total of 353 samples in Breast Tanner stages 0 - 5 were evaluated in US LH ELISA using Analyse-It<sup>®</sup> for Microsoft Excel as seen in table below.

Breast Tanner Stage	No of specimens (n)	Median Conc. (mIU/mL)	LH (mIU/mL) 95% CI
0	11	0.023	0.014 - 0.1
1	110	0.05	0.014 - 0.2
2	51	0.3	0.02 - 6.2
3	58	3.4	0.15 - 44.5
4	53	6.2	0.8 - 29.5
5	70	7.1	0.9 - 49.0

**NOTE:** It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

**REFERENCES**

1. HHS Publication, 5th ed., 2007. Biosafety in Microbiological and Biomedical Laboratories. Available <http://www.cdc.gov/biosafety/publications/bmbl5/BMML5>
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4. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

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