AnshLabs®

AnshLite™ Luteinizing Hormone CLIA RUO

AL-288

INTENDED USE

The AnshLite™ Luteinizing Hormone (LH) immunosorbent assay (CLIA) kit provides materials for the quantitative measurement of Luteinizing Hormone (LH) in the serum and other biological fluids. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

PRINCIPLE OF THE TEST

The AnshLite LH CLIA™ is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and Unknown Samples 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. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. Finally, the antibody-antigen and conjugate complex bound to the well is detected by addition of a luminogenic substrate (AnshLite™ chemiluminescence substrate solution). The relative light output units (RLU) measured, is directly proportional to the concentration of LH in the samples and LH Calibrators.

MATERIALS SUPPLIED

CAL-288A - CAL-288G LH Calibrators A through G

Seven vials, 1 mL, labeled A-G containing concentrations of approximately 0 - 18 mIU/mL of LH in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at -20°C until the expiration date. Mix well and use. Aliquot and freeze in plastic vials for multiple use.

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 = 0.71 (LH WHO NIBSC code 81/535 version 2.0.)

CTR-288-I & CTR-288-II LH Controls I & II

Two vials, 1 mL, 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 -20°C until the expiration date. Mix well and use. Aliquot and freeze in plastic vials for multiple use.

Note: ***Calibrators and controls are packed separately and shipped frozen. Store unopened at -20°C until the expiration date.***

PLT-288 LH Coated Microtitration Strips

One stripholder, 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-288 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-288 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.

ALA-100A ALA-100A Solution

One bottle, 12 mL, containing a chemiluminescent substrate solution A. Store at 2-8°C until expiration date.

ALB-100B ALB-100B Solution

One vial, 75 uL, containing an oxidizing solution B. Store at 2-8°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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate Luminometer
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
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," 5th Edition, 2007[1].

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Pro-Clean 400 and Sodium azide[2] 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.[3]

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the AnshLite™ LH CLIA 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. **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.
2. **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.
3. **Substrate Solution:** Mix 1 part of AnshLite™ B in 1000 part of AnshLite™ A (for example: 12 uL of AnshLite™ B in 12 mL of AnshLite™ A). The two components should be mixed thoroughly by gentle inversion at least 60 minutes prior to use.

   NOTE: This premixed substrate solution is stable for 8 hours at 2-8°C. Mixed substrate solution should be protected from excessive heat or direct sunlight.

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 serum samples reading higher than the highest calibrator should be mixed and diluted in the Calibrator A prior to assay.*

1. Label the microtitration strips to be used.
2. Pipette 50 µL of the Calibrator, Controls and 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 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 Substrate Solution to each well using a repeater pipette.
13. Incubate the wells, shaking at 600-800 rpm on an orbital microplate shaker for 4±1 minute at room temperature. Avoid exposure to direct sunlight.
14. Read the light output of the solution in the wells within 10 minutes, using a microplate illuminometer.

NOTE: *While reading the RLU'S of the well, it is necessary to program the 0 calibrator as "**BLANK**."*

RESULTS

NOTE: *The results in this package insert were calculated by plotting the **Relative Light Units (RLU) 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.*

1. Optimum results can be obtained at incubation temperature of **23 ± 2°C**.
2. Calculate the mean RLU for each calibrator, Control, or Unknown.
3. Plot the log of the mean RLU 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.
4. Determine the LH concentrations of the Controls and unknowns from the calibration curve by matching their mean RLU readings with the corresponding LH concentrations.

5. Any sample reading higher than the highest Calibrator should be appropriately diluted with Calibrator A and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the specimen concentration obtained in the assay by a dilution factor, for diluted specimens.

LIMITATIONS

The reagents supplied in this kit are optimized to measure LH levels in human. 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[4].

The AnshLite LH CLIA 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.
- LH CLIA 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.
- The Substrate solutions should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents	Mean RLU x10⁴	Conc (mIU/mL)
	Calibrators		
A1, A2	A	1.83 (**Blank**)	0
B1, B2	B	1.06	0.013
C1, C2	C	6.37	0.063
D1, D2	D	47.35	0.41
E1, E2	E	180.39	1.7
F1, F2	F	606.53	7.0
G1, G2	G	1348.37	20.0

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 AnshLite™ LH CLIA assay, as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 mIU/mL) and low calibrator (0.022 mIU/mL), is 0.009 mIU/mL.

Imprecision:

Reproducibility of the AnshLite™ LH CLIA assay was determined in a study using four serum sample pools. The study included a total of 10 assays, three replicates of each per assay (n=30). Representative data were calculated and are presented in the following table.

Sample	Mean conc.	Within run		Between run		Total	
	(mIU/mL)	SD	%CV	SD	%CV	SD	%CV
Pool-1	0.030	0.001	4.81%	0.001	3.62%	0.002	6.02%
Pool-2	0.129	0.005	3.79%	0.004	3.35%	0.007	5.06%
Pool-3	4.789	0.104	2.17%	0.146	3.04%	0.179	3.73%
Pool-4	4.835	0.109	2.25%	0.084	1.74%	0.138	2.85%

Linearity:

Calibrator G and four serum samples containing various LH levels were diluted serially diluted in Calibrator A. The percent (%) recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (mIU/mL)	Observed Conc. (mIU/mL)	% Recovery
Cal.G	Neat Value	19		
	1:2	9.50	9.57	101%
	1:4	4.75	5.00	105%
	1:8	2.38	2.47	104%
	1:16	1.19	1.21	102%
	1:32	0.59	0.61	103%
1	Neat Value	7.73		
	1:2	3.87	4.07	105%
	1:4	1.93	2.05	106%
	1:8	0.97	1.03	106%
	1:16	0.48	0.52	107%
	1:32	0.24	0.27	110%
2	Neat Value	8.60		
	1:2	4.30	4.50	105%
	1:4	2.15	2.24	104%
	1:8	1.08	1.13	105%
	1:16	0.54	0.57	105%
	1:32	0.27	0.28	105%
3	1:4	8.74		
	1:8	4.37	4.68	107%
	1:16	2.19	2.34	107%
	1:32	1.09	1.18	108%
	1:64	0.55	0.58	107%
	1:128	0.27	0.29	107%
4	1:4	8.16		
	1:8	4.08	4.25	104%
	1:16	2.04	2.12	104%
	1:32	1.02	1.08	106%
	1:64	0.51	0.54	106%
	1:128	0.26	0.26	101%

Recovery:

Known amounts of LH were added to four 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 Conc. (mIU/mL)	Observed Conc. (mIU/mL)	% Recovery
1	4.94	5.64	5.68	101%
		6.34	6.39	101%
		7.04	7.21	102%
2	4.81	5.52	5.54	100%
		6.23	6.39	103%
		6.94	6.88	99%
3	2.28	3.12	3.09	99%
		3.95	3.85	97%
		4.79	4.68	98%
4	1.86	2.71	2.76	102%
		3.57	3.48	97%
		4.43	4.27	96%

Analytical Specificity:

This monoclonal antibody pair used in the assay does not cross-react to other closely related analytes.

Cross-Reactant	Concentration	% Cross-reactivity
Human chorionic gonadotropin (hCG)	50,000 mIU/mL	Non-Detectable
FSH (08/282)	126 mIU/mL	Non-Detectable

Interference:

When potential interferents (Hemoglobin, Bilirubin, Biotin and Intralipids) were added at least at two times their physiological concentration to control sample, LH concentration were within ± 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	7.4	7.5	2.0
	0.5 mg/mL	7.9	8.0	1.1
	0.1 mg/mL	8.1	7.7	-5.0
Hemoglobin	1 mg/mL	4.6	4.4	-5.2
	0.5 mg/mL	4.8	4.9	2.4
	0.1 mg/mL	5.2	5.1	-2.0
Bilirubin	0.66 mg/mL	5.4	5.1	-5.4
	0.2 mg/mL	7.6	7.1	-6.1
Bilirubin	0.66 mg/mL	3.3	3.3	3.0
	0.2 mg/mL	4.7	4.7	0.5
Biotin	1200 ng/mL	7.5	7.2	-3.9
	600 ng/mL	8.1	7.8	-4.5
	200 ng/mL	8.0	8.1	1.3
Biotin	1200 ng/mL	4.6	4.6	1.2
	600 ng/mL	4.5	4.5	-4.0
	200 ng/mL	5.0	5.0	-4.7
Intralipids	20 mg/mL	7.9	7.8	-1.0
	10 mg/mL	8.2	8.1	-0.6
	5 mg/mL	8.2	8.3	0.8
Intralipids	20 mg/mL	4.6	4.6	-0.3
	10 mg/mL	5.0	5.0	0.3
	5 mg/mL	5.2	5.2	1.0

Expected Values:

Cycling female serum samples (day 2-4) were analyzed using Anshlabs US LH ELISA (AL-188). 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® 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® 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/BMBL5
2. DHHS (NIOSH) Publication No. 78–127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http:// www.cdc.gov/niosh.
3. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A3. 2004. Clinical and Laboratory Standards Institute.
4. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037–1038.

This assay is intended for Research Use Only.

Manufactured by:
Ansh Labs
445 Medical Center Blvd.
Webster, TX 77598-4217, U.S.A.