AnshLabs®

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AnshLite™ Follicle-Stimulating Hormone CLIA

AL-286

INTENDED USE

The AnshLite Follicle-Stimulating Hormone (FSH) chemiluminescent assay (CLIA) kit provides materials for the quantitative measurement of dimeric Follicle-Stimulating Hormone (FSH) in human serum and plasma. It is intended for research use.

PRINCIPLE OF THE TEST

The AnshLite FSH CLIA is a quantitative three-step sandwich type immunoassay. In the first step Calibrators, Controls and Unknown Samples are added to FSH antibody coated microtiter wells and incubated. After the first incubation and washing, the wells are incubated with biotinylated FSH 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 chemiluminescence substrate 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 relative light output units (RLU) measured is directly proportional to the concentration of FSH in the samples and calibrators.

MATERIALS SUPPLIED

CAL-286A FSH Calibrator A

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

CAL-286B - CAL-286F FSH Calibrators B thru F (Lyophilized)

Five vials, labeled B-F, containing concentrations of approximately 0.2 - 25 mIU/mL of FSH in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrators B-F with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and Freeze immediately for multiple use. Avoid repeated freeze thaws.

Traceability: The AnshLite FSH calibrators are traceable to the World Health Organization International preparation NIBSC code 81/535, Version 2.0, dated 22/10/2014.

FSH calibrators = 0.98 (FSH Human Recombinant WHO 08/282, Version 3.0, Dated 28/03/2013)

CTR-286-I & CTR-286-II FSH Controls I & II (Lyophilized)

Two vials, labeled Levels I and II containing low and high FSH concentrations in protein based buffer and Pro-Clean 400. Refer to **calibration card** for exact US equivalent concentrations. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well and use after reconstitution. Aliquot and freeze immediately for multiple use. Avoid repeated freeze thaws.

PLT-283 FSH Coated Microtitration Strips

One strip holder, containing 12 strips and 96 microtitration wells with FSH 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-183 FSH 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-286 FSH Biotin Conjugate—Ready-to-Use (RTU)

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

SAR-286 FSH 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 Anshlite A Solution

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

ALB-100B AnshLite B Solution

One vial, 75 µL, 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.

CRD-286 Calibration Card

One lot specific calibration card.

MATERIALS REQUIRED BUT NOT PROVIDED

1. 96 wells plate based chemiluminescence reader.
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. Not for use in diagnostic procedures.*

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, Li-Hep, K_2EDTA Plasma are the recommended sample types.

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.

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 FSH 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. **FSH Calibrators B-F and FSH Controls I & II**: Tap and reconstitute FSH Calibrator B-F and FSH Controls I & II each with 1 mL deionized water. Solubilize, mix well, and use after reconstitution.
   NOTE: In case sensitivity below calibrator B level is desired, dilute reconstituted calibrator B as below.
   Calibrator B/2: Mix 100 µL of reconstituted Calibrator B with 100 µL of Calibrator A.
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.
4. **Substrate Solution:** Mix 1 part of AnshLite B in 1000 part of AnshLite A (for example: 12 µL 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. 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:

a) All samples reading higher than the highest calibrator should be mixed and diluted in the 0 mIU/mL reconstituted Calibrator A prior to assay.

b) In case sensitivity below calibrator B level is desired, dilute reconstituted calibrator B as below.

Calibrator B/2: Mix 100 µL of reconstituted Calibrator B with 100 µL of Calibrator A.

1. Mix 1 part of AnshLite B in 1000 part of AnshLite A (for example: 12 µL of AnshLite B in 12 mL of AnshLite A in a HDPE Amber bottle).
2. Label the microtitration strips to be used.
3. Add **100 µL** of the FSH Assay Buffer to each microtiter well using a repeater pipette.
4. Pipette **50 µL** of the reconstituted Calibrators and Controls and Unknowns to the appropriate wells.
5. 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).
6. Aspirate and wash each strip **5 times** with Washing Solution **(350 µL/per well**) using an automatic microplate washer.
7. Add **100 µL** of the US FSH Biotin conjugate RTU solution to each well using a repeater pipette.
8. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **60 minutes** at room temperature (23 ± 2°C).
9. Aspirate and wash each well 5 times (350 µL per well) with the wash solution using an automatic microplate washer.
10. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
11. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
12. Aspirate and wash each well 5 times (350 µL per well) with the wash solution using an automatic microplate washer.
13. Add **100 µL** of the substrate solution to each well using a repeater pipette. Avoid direct exposure to heat and sunlight.

14. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for **4 ± 1 min** at room temperature (23 ± 2°C).
15. Read the light output of the solution in the wells within 10 minutes, using a microplate luminometer.
    ***NOTE**: Zero calibrator should be programmed as "**Blank**" while reading the RLU.*

RESULTS

***NOTE:** The results in this package insert were calculated by plotting the **log relative light unit (RLU) data on the y-axis and log FSH 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 FSH concentrations in mIU/mL along the x-axis, using a cubic regression curve-fit.
4. Determine the FSH concentrations of the Controls and unknowns from the calibration curve by matching their mean RLU readings with the corresponding FSH concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 mIU/mL (CAL 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, if required.

LIMITATIONS

The reagents supplied in this kit are optimized to measure FSH levels in human serum or plasma. 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 FSH 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.
- FSH CLIA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for FSH controls are printed on the **Calibration card**.
- A full calibration curve, low and high-level controls, should be included in each assay.
- The substrate solution should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean RLU x 10^4	Conc (mIU/mL)
A1, A2	A	1.5 (**Blank**)	0.0
B1, B2	B/2	1.151	0.1
C1, C2	B	2.703	0.2
D1, D2	C	15.244	1.1
E1, E2	D	87.462	61
F1, F2	E	410.012	39.0
G1, G2	F	778.947	105.0

***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 assay as calculated by the interpolation of mean plus two standard deviation of 15 replicates of calibrator A (0 mIU/mL) and calibrator B (0.23 mIU/mL) is 0.018 mIU/mL.

Imprecision:

Reproducibility of the FSH assay was determined in a study using two kit controls (4 replicates per run, n=40) and five serum sample pools (3 replicates per run, n=30). The study included a total of 10 assays. 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
CI	5.73	0.23	3.99%	0.10	1.82%	0.25	4.38%
CII	43.38	1.57	3.61%	1.22	2.81%	1.98	4.57%
Pool-1	0.86	0.05	5.94%	0.01	1.58%	0.05	6.14%
Pool-2	2.83	0.07	2.49%	0.05	1.57%	0.08	2.95%
Pool-3	17.34	0.78	4.49%	0.57	3.27%	0.96	5.55%
Pool-4	27.63	1.11	4.02%	1.02	3.69%	1.51	5.46%
Pool-5	44.15	1.29	2.93%	1.14	2.59%	1.73	3.91%

Linearity:

Calibrator F and five serum samples containing various FSH 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
Calibrator F	Neat Value	100.32		
	2	50.16	50.66	101%
	4	25.08	23.09	92%
	8	12.54	12.45	99%
	16	6.27	5.97	95%
	32	3.14	2.82	90%
S1	2	61.28		
	4	30.64	31.30	102%
	8	15.32	16.54	108%
	16	7.66	7.88	103%
	32	3.83	3.86	101%
	64	1.92	1.96	102%
S2	Neat Value	23.11		
	2	11.55	12.04	104%
	4	5.78	6.10	106%
	8	2.89	2.84	98%
	16	1.44	1.45	100%
	32	0.72	0.64	88%
S3	2	76.09		
	4	38.05	39.62	104%
	8	19.02	19.62	103%
	16	9.51	9.89	104%
	32	4.76	5.02	106%
	64	2.38	2.55	107%
S4	Neat Value	7.78		
	2	3.89	4.34	112%
	4	1.95	2.09	108%
	8	0.97	1.04	106%
	16	0.49	0.48	99%
S5	Neat Value	11.71		
	2	5.86	6.61	113%
	4	2.93	3.40	116%
	8	1.46	1.66	113%
	16	0.73	0.81	110%

	32	0.37	0.34	93%

Recovery:

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

Sample	Endogenous Conc. (mIU/mL)	Expected Concentration (mIU/mL)	Observed Concentration (mIU/mL)	%Recovery
1	0.84	5.82	6.60	113%
		10.79	11.40	106%
		15.76	16.98	108%
2	0.85	5.82	6.63	114%
		10.80	11.10	103%
		15.77	17.05	108%
3	2.45	7.34	7.62	104%
		12.24	12.18	100%
		17.13	18.36	107%
4	3.09	7.95	8.49	107%
		12.82	13.21	103%
		17.68	18.75	106%
5	6.12	10.82	11.86	110%
		15.53	15.57	100%
		20.24	20.70	102%

Analytical Specificity:

The monoclonal antibody pair used in the assay detects human FSH and cross-reactivity to other closely related structure or function is listed below.

Cross-Reactant	Concentration	% Cross-reactivity
Human Chorionic Gonadotropin (hCG > 99.5% purity)	50,000 mIU/mL	Non-Detectable
Luteinizing Hormone (hLH > 95 % purity)	500 mIU/mL	0.36
Inhibin B (InhβB > 95 % purity)	11 ng/mL	Non-Detectable
Inhibin A (InhβA > 95 % purity)	5 ng/mL	Non-Detectable
Anti-Mullerian Hormone (AMH > 95 % purity)	50 ng/mL	Non-Detectable

The monoclonal antibody pair used in the assay detects Bovine, Sheep and Squirrel Monkey. The assay does not detect Canine, Goat, Equine, Mare, Ovine, Porcine, Rat and Mouse.

Interference:

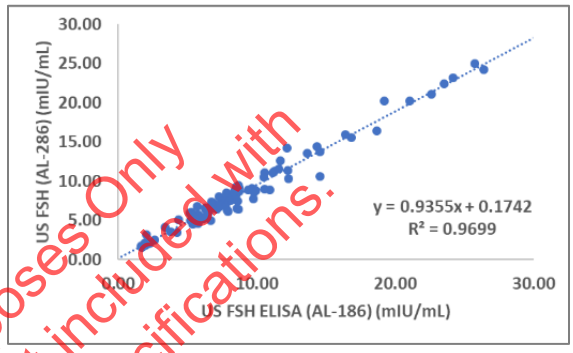
When potential interferents i.e. Hemoglobin, Bilirubin, Biotin and intralipids were added at more than twice their physiological dose to the reference samples, no significant interference was observed. The results are represented in the table below.

Interferent	Interferent Dose	Reference Sample FSH (mIU/mL)	Spiked Sample FSH (mIU/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	8.6	8.7	1.7
	0.5 mg/mL	9.1	9.1	-0.6
	0.1 mg/mL	8.9	9.4	6.5
	1 mg/mL	20.6	20.7	0.4
	0.5 mg/mL	18.8	19.0	1.1
	0.1 mg/mL	20.2	20.0	-1.1
Bilirubin	0.66 mg/mL	5.8	5.8	0.2
	0.2 mg/mL	8.1	8.1	-0.5
	0.66 mg/mL	12.9	13.6	5.3
	0.2 mg/mL	18.3	18.61	1.8
Biotin	1200 ng/mL	7.9	8.2	3.8
	600 ng/mL	9.1	9.2	1.7
	200 ng/mL	9.2	9.1	-0.6
	1200 ng/mL	18.6	18.2	-2.5
	600 ng/mL	18.6	19.3	3.6
	200 ng/mL	20.9	21.3	1.6
Intralipids	20 mg/mL	7.8	8.1	4.2
	10 mg/mL	8.4	8.8	4.0
	20 mg/mL	16.8	17.8	5.8
	10 mg/mL	19.5	20.3	4.1

Method Comparison:

The AnshLite FSH CLIA (AL-286) has been compared to Ultra-Sensitive FSH ELISA (AL-186) using 92 serum samples in the range of 1.5-25.0 mIU/mL. The Regression analysis yielded a slope of 0.94 and an intercept of 0.17mIU/ml.

US FSH (AL-286) = 0.94mIU/mL (AL-186) + 0.1742 (r=0.969)



Expected Values:

The expected ranges for FSH in pediatric male samples in the age range of 3.0 – 18.0 years were calculated using 95% non-parametric estimation. A total of 403 samples in Pubic Hair Tanner stages 1 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table below.

Pubic Hair Tanner Stage	No of specimens (n)	Median Conc. (mIU/mL)	FSH (mIU/mL) 95% CI
1	217	0.73	0.12 to 3.55
2	54	2.12	0.37 to 5.6
3	32	3.39	1.0 to 11.0
4	50	3.35	1.1 to 12.1
5	50	4.41	1.4 to 18.6

The expected ranges for FSH in pediatric female samples in the age range of 2.4 – 18.0 years were calculated using 95% non-parametric estimation. A total of 430 samples in Breast Tanner stages 0 - 5 were evaluated using Analyse-It® for Microsoft Excel as seen in table below.

Breast Tanner Stage	No of specimens (n)	Median Conc. (mIU/mL)	FSH (mIU/mL) 95% CI
0	15	1.73	1.0 to 3.8
1	173	1.84	0.3 to 5.6
2	61	3.69	0.4 to 12.5
3	58	7.44	1.3 to 28.3
4	53	8.97	1.4 to 36.1
5	70	7.97	0.5 to 16.4

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 *in vitro* research use only.

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