AnshLabs

DBS (●) Follicle-Stimulating Hormone ELISA RUO

AL-187

INTENDED USE

The Dried Blood Spot Follicle-Stimulating Hormone (FSH) immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of Follicle-stimulating hormone (FSH).

PRINCIPLE OF THE TEST

The DBS FSH ELISA 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 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 FSH in the samples and calibrators.

MATERIALS SUPPLIED

CAL-186A US FSH Calibrators A

One vial, 3 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-186B - CAL-186F US FSH Calibrators B thru F

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 US FSH calibrators are traceable to the World Health Organization International preparation NIBSC code 08/282, Version 3.0, Dated 28/03/2013.

CTR-186-I & CTR-186-II US FSH Controls I & II

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 DBS 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-183 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-186 US 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-186 US 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.

EXB-129 Extraction Buffer/Sample Diluent

One bottle, 45 mL, containing a protein-based (BSA)-buffer with a nonmercury preservative. Store at 2-8°C until expiration date.

Note: *Additional bottles of EXB-129, DBS AMH Extraction Buffer / Sample Diluent can be ordered if higher dilution is required.*

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-187 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 µL.
5. Disposable 12 x 75 mm culture tubes.
6. Tight fitting 12 x 75 mm tube racks.
7. Vortex mixer.

8. Deionized water.
9. Ahlstrom 226 or Whatman 903 (Protein Saver Card)
10. 6mm round automated puncher. For machine punching, puncher catalog number 1296-071 from PerkinElmer can be used.
11. DBS 5/16" (7.9) round puncher. For manual punching Punchline catalog number 53700 from McGill incorporated can be used.

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

Dried blood spot is the recommended sample type.

For Dried Blood Spot Specimens

a. Use with capillary blood samples collected and dried on filter paper according to the standard procedures established for blood collection on filter paper.

b. Wipe away the first blood drop and apply surface of the first filter paper circle to the next large drop of blood, allowing the blood to fill and completely saturate the circle.

c. Never use the front as well as back of the paper to fill the circle.

d. Fill at least two circles and if possible, all circles with blood.

e. After collection, dry the blood impregnated filter papers for 2- 4 hours in a horizontal position at room temperature.

f. The dried filter paper blood spots should be stored in a low permeability re-sealable pouch at 2-8°C for up to 1 week or frozen at -20°C or lower for up to 3 months.

g. The use of desiccant and vacuum packing to protect from moisture is highly recommended.

PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the DBS FSH 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. **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 for 10 minutes and mix well. Solubilize again for 10 minutes and mix well before using.
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.

DBS EXTRACTION PROCEDURE

Extraction of FSH from dried blood spots should be performed on the same day prior to testing.

NOTE: All blood spots should be inspected for quality.

- Do not use spots if the circle is not filled and impregnated with blood.
- Do not use irregular shaped spots, spots that are not impregnated throughout, or spots with multiple spotting.
- Do not use spots that have not been properly dried.

1. Label two 12 X 75 culture tubes for each unknown dried blood sample.
2. Punch out **two filter paper discs (7.9 mm) or four filter paper discs (6mm),** impregnated with the unknown dried blood specimen, onto a clean surface and transfer the discs using clean tweezers into the corresponding tube.
3. Alternatively, punch out the paper disc directly into the culture tube using the commercially available automated punchers.
4. Add **450 µL of the Extraction Buffer** to each tube, vortex well.
5. Place the tubes in a tight-fitting tube rack and incubate the tubes, shaking at a slow speed (500 - 600 rpm) at room temperature for 60 minutes.
6. Transfer the liquid from one tube into the corresponding second labeled tube. Leave the blood spot in the initial tube.
7. The blood extract is now ready for analysis.
8. The extracted sample (without the extracted blood spot) is stable for up to 7 days at -20°C.
9. Use the **calibrator assignment for two spots** as mentioned in the calibration card for plotting the calibration curve.

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.

Protocol-1: DBS Samples

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

1. Label the microtitration strips to be used.
2. Pipette **25 µL** of the reconstituted Calibrators and Controls to the appropriate wells and add **75 µL** of the FSH Assay Buffer to calibrators and controls wells using a repeater pipette.
3. Pipette **100 µL** of the extracted DBS samples (see DBS extraction procedure) to the appropriate wells. **Note:** ***Do not add FSH Assay Buffer to the extracted sample wells.***
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 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 FSH 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 **30 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.***

Protocol-2: Serum Samples

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

1. Label the microtitration strips to be used.
2. Add 100 µL of the FSH Assay Buffer to each microtiter well using a repeater pipette.
3. Pipette 25 µL of the reconstituted Calibrators and Controls and unknowns to the appropriate wells.
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 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 FSH 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 **30 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.***

Protocol for Dynex Technology (DS2)

Protocol for Dynex DS2 can be provided upon request.

RESULTS

NOTE: *The results in this package insert were calculated by plotting the **log optical density (OD) 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 OD for each calibrator, Control, or Unknown.
3. Plot the log of the mean OD 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 OD 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 DBS. 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 DBS FSH 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.
- DBS FSH ELISA 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.
- 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	0.037 (Blank)	0
B1, B2	B	0.032	1.0
C1, C2	C	0.12	4.0
D1, D2	D	0.45	16.0
E1, E2	E	1.52	65.0
F1, F2	F	3.26	160.0

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 deviations of 16 replicates of calibrator A (0 mIU/mL) and calibrator B (1.19 mIU/mL) is 0.12 mIU/mL.

Imprecision:

Reproducibility of the assay was determined in a study using two kit controls and five serum sample pools. The study included a total of 10 assays, three replicates of each per assay (n=40). Representative data were calculated and are presented in the following table.

Sample ID	Mean Conc.	Within run		Between run		Total	
		SD	%CV	SD	%CV	SD	%CV
CI	1.32	0.05	4.07%	0.07	5.41%	0.09	6.77%
CII	7.16	0.32	4.45%	0.12	1.68%	0.34	4.75%
Pool-1	1.56	0.09	5.90%	0.07	4.67%	0.12	7.52%
Pool-2	3.10	0.25	7.98%	0.12	3.99%	0.28	8.92%
Pool-3	4.39	0.21	4.87%	0.16	3.68%	0.27	6.10%
Pool-4	6.93	0.37	5.29%	0.29	4.11%	0.46	6.69%
Pool-5	8.98	0.39	4.33%	0.55	6.08%	0.67	7.46%

Linearity:

Calibrator F and three 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
Cal F	Neat Value	26.00		
	2	13.00	13.18	101%
	4	6.50	6.62	102%
	8	3.25	3.19	98%
	16	1.63	1.58	97%
	32	0.81	0.81	99%
1	Neat Value	8.82		
	2	4.41	4.47	101%
	4	2.21	2.21	100%
	8	1.10	1.04	94%
	16	0.55	0.52	94%
	32	0.28	0.26	95%
2	Neat Value	2.87		
	2	1.43	1.41	98%
	4	0.72	0.68	95%
	8	0.36	0.35	97%
	16	0.18	0.16	90%
3	2	18.34		
	4	9.17	9.51	104%
	8	4.58	4.90	107%
	16	2.29	2.33	102%
	32	1.15	1.18	103%
	64	0.57	0.59	103%

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	2.75	3.80	3.89	102%
		4.85	4.91	101%
		5.91	5.91	100%
2	2.98	4.02	4.69	117%
		5.06	5.31	105%
		6.10	6.89	113%
3	5.94	6.83	6.52	95%
		7.72	7.67	99%
		8.62	8.63	100%
4	0.95	2.09	2.17	104%
		3.23	3.40	105%
		4.38	4.72	108%
5	1.50	2.62	2.53	97%
		3.73	3.75	101%
		4.85	4.84	100%

Analytical Specificity:

The monoclonal antibody pair used in the assay detects human FSH and does not cross-react with hLH and hCG. Antibody pair used in the assay detects Bovine, Canine, Mouse, and Goat.

Cross-Reactant	Concentration	% Cross-reactivity
Human chorionic gonadotropin (hCG)	50,000 mIU/mL	Non-Detectable
Luteinizing Hormone (hLH)	500 mIU/mL	Non-Detectable

Hook Effect:

There is no high-dose hook effect at FSH concentrations up to 80.5 mIU/mL.

Interference:

When potential interferents (Hemoglobin, Bilirubin, Biotin and Intralipids) were added at twice their physiological dose to the control samples, no significant interference was observed. The results are represented in the table below.

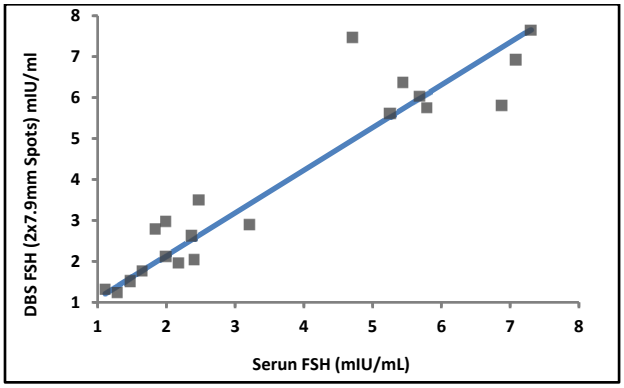
Interferent	Interferent Dose	Sample FSH (mIU/mL)	Dosed Sample FSH (mIU/mL)	% Difference to Reference
Hemoglobin	1 mg/mL	1.0	3.9	-5.1
	0.5 mg/mL	4.4	4.2	-3.1
	0.1 mg/mL	4.5	4.5	-1.7
Hemoglobin	1 mg/mL	3.6	3.5	-4.3
	0.5 mg/mL	3.6	3.5	-1.2
	0.1 mg/mL	3.9	3.8	-3.0
Bilirubin	0.66 mg/mL	2.5	2.8	11.7
	0.2 mg/mL	4.1	4.1	-1.9
Bilirubin	0.66 mg/mL	3.4	3.2	-7.6
	0.2 mg/mL	5.0	4.97	-1.0
Biotin	1200 ng/mL	5.7	5.8	0.6
	600 ng/mL	5.9	5.8	-2.0

	200 ng/mL	6.1	6.2	0.7
Biotin	1200 ng/mL	4.9	4.8	-1.3
	600 ng/mL	5.3	5.2	-1.0
	200 ng/mL	5.2	5.0	-4.4
Intralipids	20 mg/mL	5.0	5.4	8.7
	10 mg/mL	5.6	5.6	-1.2
Intralipids	20 mg/mL	4.2	4.4	4.6
	10 mg/mL	4.4	4.7	7.3

Method Comparison:

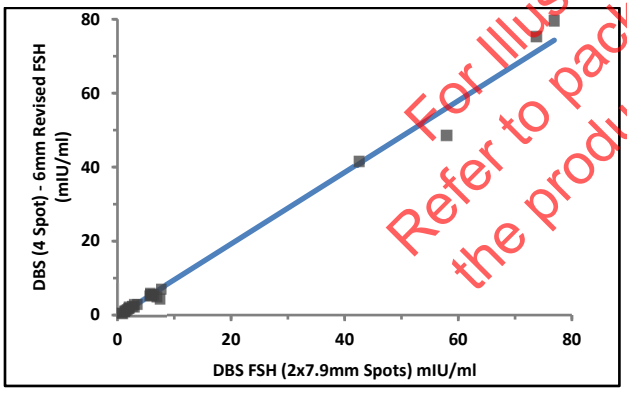
The DBS FSH samples has been compared to serum FSH samples using 20 matched serum and dried blood spot samples. Passing Bablok analysis of the results yielded the following regression:

DBS FSH = 0.061 + 1.042 Serum FSH, r=0.939



The DBS FSH (4x6mm spots) samples has been compared to DBS FSH (2x7.9mm) samples using 25 matched samples using calibration concentration specified in the calibration Card (CD-186). Passing Bablok analysis of the results yielded the following regression:

DBS FSH (4x6mm Spots) = -0.1152 + 0.9685 DBS FSH (2x7.9mm Spots) mIU/ml, r=0.996



Expected Values:

Cycling female serum samples (day 2-4) were analyzed using US FSH 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 FSH conc. (mIU/mL)	FSH Range (mIU/mL)
24-29	13	7.2	3.7 – 11.4
30-35	34	7.5	3.1 – 11.5
36-39	22	8.8	1.5 – 41.5
40-43	24	7.1	1.4 – 20.1

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 *Research Use Only*.

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