PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

1. Accuracy: In an internal study **Electra™ FSH** was evaluated against commercially available licensed kit with 90 random clinical samples & **Electra™ FSH** has demonstrated >98% clinical correlation with the commercially available licensed kit.
2. Precision: **Electra™ FSH** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with Electra™ FSH	Coefficient of Variable (CV)
Level 1	10	8.32	4.87
Level 2	10	20.22	4.48
Level 3	10	45.96	5.42

B) External Evaluation:

Electra™ FSH CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ FSH** has demonstrated 97% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd).

Important Note:

1. The **Electra™ FSH** assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 22°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipette is available.
4. Duplication of Standards & Samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

(1). As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. (2).The activity of the enzyme used is temperature-dependent and the RLU values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the RLU values. Corresponding variations apply also to the incubation times. However, the Standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result. (3). Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits. (4).Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect results.

BIBLIOGRAPHY

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2. Cohen K.L. Metabolism 1977; 26:1165.
3. Rebar W.R. Erickson G.F. and Yen S.S.C Fertil. Steril. 1982 37:35.
4. Abraham G.E. Ed. Radioassay systems in Clinic. Endocrinol. Marcel Dekker, Inc New York (1981).
5. Uotila M, Ruoslahti and Engavall E.J. Immunol Methods 1981 42:11.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:

Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa- 403 722, INDIA.

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An ISO 13485 Certified Company

0320/VER-01

electra™ FSH

Chemiluminescence Assay for the Quantitative Determination of Follicle-Stimulating Hormone (FSH) in Human Serum.

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

ELECTRA™ FSH CLIA test is intended for the quantitative determination of Follicle-Stimulating Hormone (FSH) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback relationship. FSH is a glycoprotein secreted by the basophilic cells of the anterior pituitary. Gonadotropin-release hormone (GnRH), produced in the hypothalamus, controls the release of FSH from anterior pituitary. Like other glycoproteins, such as LH, TSH, and HCG, FSH consists of subunits designated as alpha and beta. Hormones of this type have alpha subunits that are very similar structurally therefore the biological and immunological properties of each are dependent on the unique beta subunit. In the female, FSH stimulates the growth and maturation of ovarian follicles by acting directly on the receptors located on the granulosa cells; follicular steroidogenesis is promoted and LH production is stimulated. The growth of the seminiferous tubules and maintenance of spermatogenesis in men are regulated by FSH.Tumors of the testes generally depress serum FSH concentrations. High levels of FSH in men may be found in primary testicular failure and Klinefelter syndrome. Elevated concentrations are also present in cases of starvation, renal failure, hyperthyroidism, and cirrhosis.

PRINCIPLE

ELECTRA™ FSH Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ FSH CLIA** works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.

The **ELECTRA™ FSH** Quantitative Test Kit is based on a solid phase enzyme immunoassay. The assay system utilizes one anti-FSH antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-FSH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with Wash Buffer to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and Luminescence is measured in RLU. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of FSH in the sample. By reference to a series of FSH standards assayed in the same way, the concentration of FSH in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Anti- FSH antibody.
- FSH HRPO Enzyme Conjugate
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- FSH Standard set of 6 standards labeled as A to F in liquid form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided:

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

STORAGE AND STABILITY

1. **ELECTRA™ FSH** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. In case the desiccant pouch changes color from blue to white, the strips should not be used.

3. Diluted wash buffer is stable up to one week at 2-8°C.
4. Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION

1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

- All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
- Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate-A µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate-B µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **25**µl of Standards & Serums into the appropriate wells.
2. Dispense **50**µl of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **30 minutes**.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto the absorbent paper or paper towels to remove all residual water droplets.
4. Add **50** µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
5. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (18-25°C) in dark.
6. Read the **ELECTRA™** micro-plate exactly at **10** minutes in **ELECTRA ™ Analyzer.** If **ELECTRA™** micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

Add **25** µl Standards/Samples into the respective Microwell

↓

Add **50** µl Enzyme Conjugate in each well

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Apply plate sealer and incubate for **30** minutes at **18-25°C**

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Wash Microwells **5 times** with 350 µl **of** diluted Wash Buffer

↓

Add **50** µl Substrate in each well

↓

Incubate for **10** Minutes at **18-25°C in dark**

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Read results on **ELECTRA ™** Analyzer exactly at the end of **10** Minutes

CALCULATIONS

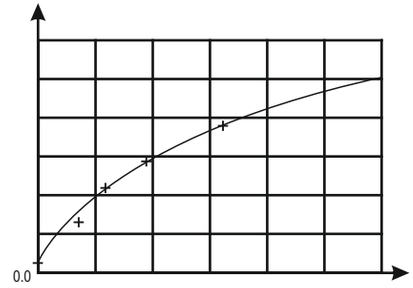
Construct a standard curve by plotting the RLU obtained from each reference standards against its Concentrations in mIU/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of FSH in mIU/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with RLU's shown in the Y axis against FSH concentrations shown in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

FSH Values (mIU/ml)	RLU's
A	41
B	14832
C	82432
D	447660
E	885483
F	1848339



This Standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own Standard curve and data.

Expected Ranges of values

It is important for each laboratory to establish the normal range limits. The following normal range should be considered as guideline only:

Female Follicular	0~20 mIU/ml
Mid-cycle	15~30 mIU/ml
Luteal	0~20 mIU/ml
Post Menopausal	40~200 mIU/ml
Male	0~20 mIU/ml

The minimal detectable concentration of human FSH by this assay is estimated to be 2.5mIU/ml.