

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

- Accuracy: In an internal study **Electra™ β-hCG** was evaluated against commercially available licensed kit with 90 random clinical samples & **Electra™ β-hCG** has demonstrated 100% clinical correlation with the commercially available licensed kit.
- Precision: **Electra™ β-hCG** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with Electra™ β-hCG	Coefficient of Variable (CV)
Level 1	10	1.70	7.25
Level 2	10	8.26	5.72
Level 3	10	72.00	4.93

B) External Evaluation:

Electra™ β-hCG CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ β-hCG** has demonstrated 98% correlation with the reference method.
*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd).

Important Note:

- The **Electra™ β-hCG** assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 22°C.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- 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.
- 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

(1). Swaminathan, N. and Bahl, O.P., Biochem. Biophys. Res. Commun., 40, 422-427 (1970). (2). Morgan, F.J. and Canfield, R.E., Endocrinology, 88, 1045-1053. (3). Bahl, O.M., Carlsen, R.B., Bellisario, R. and Swaminathan, N., Biochem. Biophys. Res. Commun., 48, 416-422 (1972). (4). Bellisario, R., Carlsen, R.B. and Bahl, O.P., J. Biol. Chem., 248, 6796-6809 (1973). (5). Morgan, F.J., Canfield, R.E., Vaitukaitis, J.L. and Ross, G.T., Endocrinology, 94, 1601-1606 (1974). (6). Morgan, F.J., Birken, S. and Canfield, R.E., J. Biol. Chem., 250, 5247-5258 (1975). (7). Lenton, E.A., Neal, L.M. and Sulaiman, R. Fertility and Sterility, 37, 773-778 (1982). (8). Batzer, R.F., Fertility and Sterility, 37, 1-13 (1980). (9). Braunstein, G.D., Rasor, J., Adler, D., Danzer, H. and Wade, M.E., AmJ. Obstet. Gynecol., 126, 678-681 (1976). (10). Catt, K.J., Dufau, M.L. and Vaitukaitis, J.L., J. Clin. Endocrinol. Metab. 40, 537-540 (1975). (11). Kosasa, T., Levesque, L., Goldstein, D.P. and Taymor, M.L., J. Clin. Endocrinol. Metab., 36, 622-24 (1973). (12). Braunstein, G.D., Vaitukaitis, J.L., Carbone, P.P. and Ross, G.T., Ann. Intern. Med., 78, 39-45 (1973).

SYMBOL KEYS

 Temperature Limitation	 Consult Instructions for use	 Date of Manufacture	 Batch Number / Lot Number
 Manufacturer	 In vitro Diagnostic Medical Device	 This side up	 Contains sufficient for <n> tests
 Use by	 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.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

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0320/VER-01

electra™
β-hCG

Chemiluminescence Assay for the Quantitative Determination of Beta-Human Chorionic Gonadotropin (β-hCG) in Human Serum.
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE

ELECTRA™ β-hCG CLIA test is intended for the quantitative determination of Beta-Human Chorionic Gonadotropin (β-hCG) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Human chorionic gonadotropin (hCG) is a sialoglycoprotein. hCG is initially secreted by the trophoblastic cells of the placenta shortly after implantation of the fertilized ovum into the uterine wall. The rapid rise in hCG serum levels after conception makes it an excellent marker for early confirmation and monitoring of pregnancy. Physiologically, hCG appears to maintain the corpus luteum, thereby allowing synthesis of progesterone and estrogens that support the endometrium. As uncomplicated pregnancies progress, the placenta assumes the production of these hormones. The serum hCG levels increase to a peak concentration, then decrease and plateau. hCG circulates as the intact molecule in the serum of normal women who have an uncomplicated pregnancy. The subunits are cleared rapidly and excreted by the kidney. The placental hormone hCG, is similar to luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). All are glycoproteins consisting of two non-covalently bound dissimilar subunits, designated alpha and beta, with attached carbohydrate sidechains. The alpha subunits of these glycoproteins are very similar. In contrast, the beta subunit portions determine the biological and immunochemical specificities. The beta subunits of hCG and LH exhibit considerable homology in amino acid content. Amino acid residues specific for the beta subunit of hCG confer the immuno-chemical specificity. With the availability of sensitive quantitative assays for the measurement of serum β-hCG, it has been shown that hCG levels can be useful in predicting spontaneous abortions, aiding in the detection of ectopic pregnancy and multiple gestation. Elevated levels of hCG have also been detected in serum from patients with abnormal physiological conditions not related to pregnancy.

PRINCIPLE

ELECTRA™ β-hCG Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ β-hCG 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™ β-hCG** Quantitative Test Kit is based on a solid phase enzyme immunoassay. The assay system utilizes one anti-βhCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-β-hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the anti-β-hCG antibody coated microtiter wells and incubated with the Zero Buffer. If antigen is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen and conjugate (Anti-β-hCG antibody labeled with horseradish peroxidase) is added. The conjugate will bind immunologically to the β-hCG on the well, resulting in the antigen 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 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 Total β-hCG in the sample. By reference to a series of Total β-hCG standards assayed in the same way, the concentration of Total β-hCG in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with Anti-β-hCG antibody.
- β-hCG Zero Buffer. Ready to use.
- β-hCG Enzyme Conjugate. Ready to use
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- β-hCG 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

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Chemiluminescence assay

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- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

STORAGE AND STABILITY

1. **ELECTRA™ β-hCG** 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.

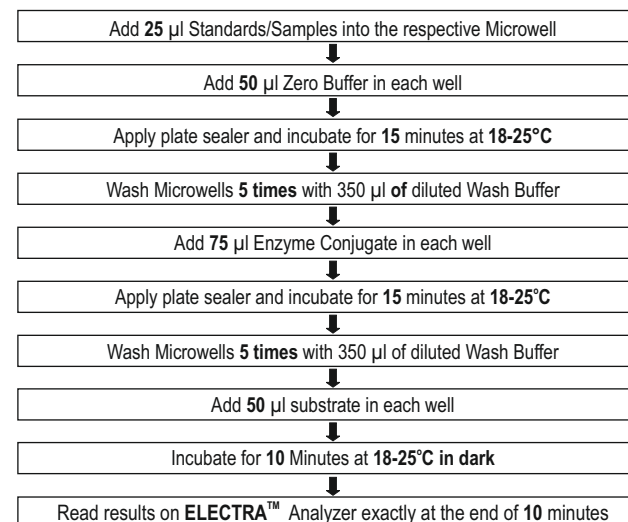
REAGENT PREPARATION

- 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 Zero Buffer into each well. Incubate at room temperature (**18-25°C**) for **15 mins**
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 absorbent paper or paper towels to remove all residual water droplets.
4. Dispense **75 µl** of Enzyme Conjugate reagent into each well. Incubate at room temperature **18-25°C** for **15 mins**.
5. 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 absorbent paper or paper towels to remove all residual water droplets.
6. Add **50 µl** of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate. Cover the **ELECTRA™** microplate and incubate for 10 minutes at room temperature (**18-25°C**) in dark.
7. 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.



CALCULATIONS

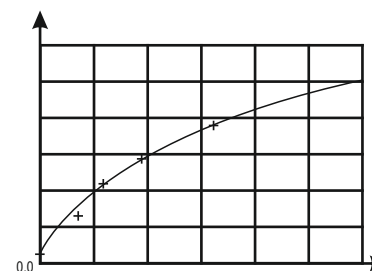
Construct a standard curve by plotting the RLU obtained from each reference standards against its concentrations in mIU/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of β-hCG 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 β-hCG concentrations shown in the X axis.

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

β-hCG Values (mIU/ml)	RLU's
A	2652
B	52799
C	372563
D	1122761
E	3136302
F	5238888



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

Each laboratory must establish its own normal ranges based on patient population. Total β-hCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The concentration of β-hCG in the serum of pregnant women increases to 5-50 mIU/ml one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 100,000-200,000 mIU/ml at the end of the first trimester. The minimum detectable concentration of β-hCG by this assay is estimated to be 2.0 mIU/ml.