random clinical samples, & **ELECTRA ™ fT4** has demonstrated 100% clinical correlation with the commercially available licensed kit.

2. Precision: **ELECTRA ™ fT4** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

| Controls | No. of testings | Mean Control values with ELECTRA ™ fT4 | Coefficient of Variable (CV) |
|---|---|---|---|
| Level 1 | 10 | 0.804 | 7.53 |
| Level 2 | 10 | 2.236 | 5.74 |
| Level 3 | 10 | 2.845 | 6.44 |

**B) External Evaluation:**

**ELECTRA™ fT4** CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **ELECTRA ™ fT4** has demonstrated 100% correlation with the reference method.

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

**Important Note**:

1. The **ELECTRA ™ fT4** 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 RLU values.

## BIBLIOGRAPHY

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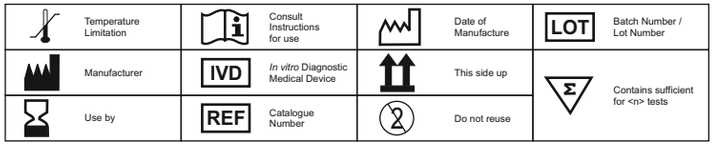
**SYMBOL KEYS**




Manufactured by:

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**An ISO 13485 Certified Company**

0320/VER-01

# electra™ fT4

**Chemiluminescence Assay for the Quantitative Determination of Free Thyroxine (fT4) in Human Serum.**
**FOR IN VITRO DIAGNOSTIC USE ONLY**
**Store at 2°C to 8°C**

## INTENDED USE

**ELECTRA™ fT4** CLIA test is intended for the quantitative determination of Free Thyroxine (fT4) in human serum. For In Vitro Diagnostic Use only.

## INTRODUCTION

Thyroxine, the principle thyroid hormone, circulates in blood almost completely bound to carrier proteins. The main carrier is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of thyroxine is responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total thyroxine level changes so that the free thyroxine concentration remains constant. Thus, measurements of free thyroxine concentrations correlate better with clinical status than total thyroxine levels.

## PRINCIPLE

**ELECTRA™ fT4** Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ fT4 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.

In **ELECTRA™ fT4 CLIA** Standard & patient specimen is first added to a microplate well. Enzyme-T4 conjugate is added, then the reactants are mixed. A competition reaction results between the enzyme conjugate and the free thyroxine for a limited number of antibody combining sites immobilized on the well. After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate by aspiration or decantation. 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 inversely related to the amount of unlabeled fT4 in the sample. By reference to a series of fT4 standards assayed in the same way, the concentration of fT4 in the unknown sample is quantified.

## MATERIALS & COMPONENTS

**Materials provided with the test kits:**

- Coated Microwells: Microwells coated with Anti- fT4 antibody.
- fT4 HRPO Conjugate Diluent
- fT4 HRPO Enzyme Conjugate (20X)
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- fT4 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, 50-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA ™** Analyzer.

## STORAGE AND STABILITY

1. **ELECTRA™ fT4** 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.
- Dilute Enzyme Conjugate with Conjugate Diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.

| No. of Strips | 0.5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $fT_4$ HRPO Enzyme Conjugate (20X) (µl) | 12.5 | 25 | 50 | 75 | 100 | 125 | 150 | 175 | 200 | 225 | 250 | 275 | 300 |
| $fT_4$ HRPO Conjugate Diluent (µl) | 250 | 500 | 1000 | 1500 | 2000 | 2500 | 3000 | 3500 | 4000 | 4500 | 5000 | 5500 | 6000 |

3. 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 diluted 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 diluted conjugate in each well

↓

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

↓

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**

↓

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

## CALCULATIONS

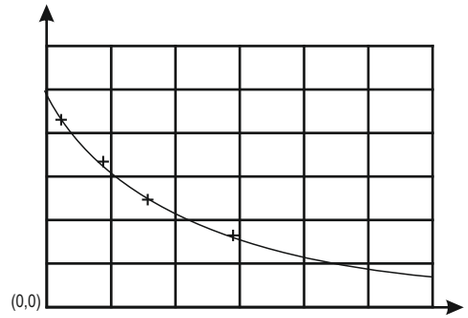
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in ng/dl on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of $fT_4$ in ng/dl 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 $fT_4$ concentrations shown in the X axis.

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

| $fT_4$ Values (ng/dl) | RLU's |
|---|---|
| A | 2915670 |
| B | 2890640 |
| C | 2133792 |
| D | 1321987 |
| E | 690452 |
| F | 394185 |




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**

A study of euthyroid adult population was undertaken to determine expected values for the Free T4 Chemiluminesence System. The mean (X) values, standard deviations (SD) and expected ranges (±2 SD) are presented below:

| | Normal Adult (90 specimens) | Pregnancy (50 specimens) |
|---|---|---|
| Mean (X) | 1.31 | 1.46 |
| Standard Deviation (SD) | 0.33 | 0.36 |
| Expected Ranges (±2 SD) | 0.65 – 1.97 | 0.61 – 2.09 |

## PERFORMANCE CHARACTERISTICS

**A) Internal Evaluation:**

1. Accuracy: In an internal study **ELECTRA ™ $fT_4$** was evaluated against commercially available licensed kit with 90