

random clinical samples, & **ELECTRA™ T4** has demonstrated 100% clinical correlation with the commercially available licensed kit.

2. Precision: **ELECTRA™ T4** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testings	Mean Control values with ELECTRA™ T4	Coefficient of Variable (CV)
Level 1	10	5.414	4.16
Level 2	10	11.257	4.83
Level 3	10	12.912	4.46

#### B) External Evaluation:

**ELECTRA™ T4** CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **ELECTRA™ T4** has demonstrated > 97% correlation with the reference method.

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

#### Important Note:

- The **ELECTRA™ T4** 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). Rongen HA, Hoetelmans RM, Bult A, van Bennekomp WP. Chemiluminescence and immunoassays. **J Pharm Biomed Anal** 1994 Apr;12(4):433-62. (2). Koszegi T, Immunoluminometric detection of human prolactin. **J Biochem Biophys Methods** 2002 Oct;53(13):157-64. (3). Roda A, Simoni P, Mirasoli M, Baraldini M, Violante FS. Development of a chemiluminescent enzyme immunoassay for urinary 1-hydroxypyrene. **Anal Bioanal Chem** 2002 Apr;372(7-8). (4). Skelley, D., Brown, L., and Besch, P. Radioimmunoassay. **Clin. Chem.** 19:146;1973. (5). Wistom, G.B. Enzyme-Immunoassay. **Clin. Chem.** 22: 1243; 1976. (6). Schuur, A.H.W.M. and Van Weeman, B.K. Review, Enzyme immunoassay. **Clin. Chem. Acta.** 81:1; 1977. (7). Ravel, R. **Clinical Laboratory Medicine**. Year Book Medical Publ. Chicago. (1973). (8). Robbins, J. Radioassay and Thyroid Gland. **Metabolism** 22:1021; 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.

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Certified Company

1119VER-01



**Chemiluminescence Assay for the Quantitative Determination of Thyroxine (T4) in Human Serum.**  
**FOR IN VITRO DIAGNOSTIC USE ONLY**  
Store at 2°C to 8°C

#### INTENDED USE

**ELECTRA™ T4** CLIA test is intended for the quantitative determination of Thyroxine (T4) in human serum. For In Vitro Diagnostic Use only.

#### INTRODUCTION

L-Thyroxine (T4) is a hormone that is synthesized and stored in the thyroid gland. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Measurement of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in patients. Increased levels of T4 have been found in hyper-thyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroiditis (Hashimoto's disease) and with some genetic abnormalities.

#### PRINCIPLE

**ELECTRA™ T4** Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ T4 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  $\lambda=425\text{nm}$ .

In **ELECTRA™ T4 CLIA**, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, T4 in the samples and conjugated T4 compete for the limited binding sites on the anti-T4 antibody of the wells. After incubation, the wells are washed by wash Buffer to remove unbound T4 conjugate. 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 T4 in the sample. By reference to a series of T4 standards assayed in the same way, the concentration of T4 in the unknown sample is quantified.

#### MATERIALS & COMPONENTS

##### Materials provided with the test kits:

- Coated Microwells: Microwells coated with Anti- T4 antibody.
- T4 HRPO Conjugate Diluent
- T4 HRPO Enzyme Conjugate (20X)
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- T4 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

- ELECTRA™ T4** kit is stable at 2-8°C up to the expiry date printed on the label.
- 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.
- Diluted wash buffer is stable up to one week at 2-8°C.
- Working Substrate (A+B) must be used immediately.

#### SPECIMEN COLLECTION

- Collect blood specimen by venipuncture according to the standard procedure.
- Only serum should be used.



Chemiluminescence assay



Chemiluminescence assay



Chemiluminescence assay



Chemiluminescence assay

- Avoid grossly hemolytic, lipemic or turbid samples.
- Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
- For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
- Do not heat inactivate before use.
- Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
- Specimen should be free from particulate matter and microbial contamination

#### PRECAUTIONS

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

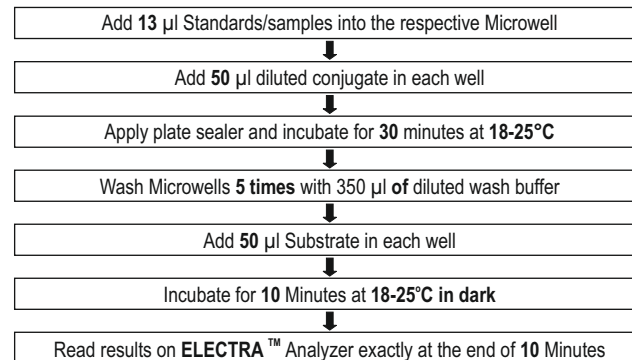
No. of Strips	0.5	1	2	3	4	5	6	7	8	9	10	11	12
T <sub>4</sub> HRPO Enzyme Conjugate (20X) (μl)	12.5	25	50	75	100	125	150	175	200	225	250	275	300
T <sub>4</sub> HRPO Conjugate Diluent (μl)	250	500	1000	1500	2000	2500	3000	3500	4000	4500	5000	5500	6000

- 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

- Secure the desired number of coated wells in the holder. Dispense 13μl of Standards & Serums into the appropriate wells.
- Dispense 50μl of diluted Conjugate into each well. Incubate at room temperature (18-25°C) for 30 minutes.
- 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.
- 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.
- 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

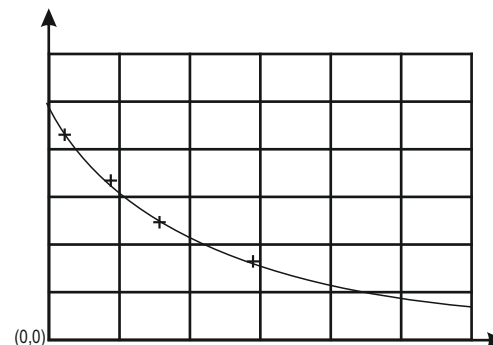
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in μg/dl on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of T<sub>4</sub> in μg/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 T<sub>4</sub> concentrations shown in the X axis.

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

T <sub>4</sub> Values (μg/dl)	RLU's
A	2302789
B	1157452
C	634237
D	322237
E	233875
F	36312



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

Normal Range: 5.0 to 13.0 μg/dl

The minimal detectable concentration of T<sub>4</sub> by this assay is estimated to be 0.45 μg/dl.

#### PERFORMANCE CHARACTERISTICS

##### A) Internal Evaluation:

- Accuracy: In an internal study **ELECTRA™ T<sub>4</sub>** was evaluated against commercially available licensed kit with 90