# DRG® Total T4 ELISA (EIA-4568)

**Revised 20 Nov. 2010 rm (Vers. 3.1)** **RUO in the USA**

***Please use only the valid version of the package insert provided with the kit.***

***This kit is intended for Research Use Only. Not for use in diagnostic procedures.***

## 1 PRINCIPLE

Competitive Enzyme Immunoassay.

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites.

After equilibrium is attained, the antibody bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 2 REAGENTS

Each kit contains reagents sufficient for 96/48 determinations

1. **Microtiter plate**:
   12 modules with 8 wells each= 96 determinations
2. T4-**Standards**: ready-to-use reagents (0.2 mL), 6 vials,
   concentrations: see labels.
   Preservative: Thimerosal 0.01% Kathon 0,1%
3. **Peroxidase Enzyme Conjugate**
   1 x 12 mL, ready to use
4. **Washing buffer** (40 fold concentrate) : 1x 25 mL
5. **TMB–Substrate** ready-to-use reagent: 1 x 12 mL
6. **Stop Solution** ready to use: 1 x 14 mL,
   contains 0.5 M $H_2SO_4$
   Avoid contact with the stop solution. It may cause skin irritations and burns.

## 3 STORAGE AND STABILITY

Store the kit at 2 °C - 8 °C.

For expiry date of the original packed kit see kit label

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## 4 PRECAUTIONS

1. Do not pipette by mouth
2. Standards are of human origin. These materials have been classified as HIV and HbsAg negative. But for safety reasons all reagents of human origin should be handled as if potentially infectious.

## 5 MATERIAL REQUIRED BUT NOT PROVIDED

1. Precision micropipettes (volume: 10 µL and 100 µL) with disposable tips
2. Distilled water
3. ELISA photometer with 450nm- and 620-655nm-filters

## 6 ASSAY PROCEDURE

1. Prior to use bring all reagents to room temperature
2. Prior dilutions :
   Dilute 25 mL of concentrated Wash Solution with 975 mL deionized water to a final volume of 1000 mL.
3. Pipette 10 µL of e.g. standards or samples into each well.
   Pipette all standards and samples within 10 minutes !
4. **Incubate 5 minutes at room temperature.**
5. Add 100 µL peroxidase conjugate into each well.
6. Mix by moving plate on the table (10sec)
7. **Incubate 80 minutes at room temperature ( 18-26°C)**
8. Remove solution from the wells by aspirating or tapping the plate
9. For washing fill plate with Washing buffer and remove; repeat wash 4x.
10. Pipette 100 µL TMB-substrate solution into each well
11. Incubate **10 min** at room temperature (**18-25°C)**
    Incubate **7 min** at room temperature (**26-29°C)**
    Incubate **5 min** at room temperature **(more than 29°C)**
12. Add 100 µL stop solution into each well(same order as substrate solution)

**Results**

The absorbance of the samples is measured with a conventional ELISA-Reader at 450 nm against air (reference wavelength 620-655 nm).

The concentrations of T4 can be calculated automatically by using an ELISA software or manually by plotting the standard concentrations over their absorbances in a lin-lin plot.

T4 concentrations of the samples can be calculated directly from the standard curve.

## 7 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## 8 RESULTS

A dose response curve is used to ascertain the concentration of thyroxine in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding T4 concentration in nmol/L on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of T4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in nmol/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 0.82 (intersects the standard curve at (87 nmol/L) T4 concentration (See Figure 1).

EXAMPLE 1

| WELL | SERUM REFERENCES | ABSORBANCE |
|---|---|---|
| 1 | 0.0 nmol/L | 2.06 |
| 2 | 0.0 nmol/L | 2.03 |
| 3 | 25 nmol/L | 1.78 |
| 4 | 25 nmol/L | 1.74 |
| 5 | 50 nmol/L | 1.23 |
| 6 | 50 nmol/L | 1.19 |
| 7 | 100 nmol/L | 0.62 |
| 8 | 100 nmol/L | 0.64 |
| 9 | 175 nmol/L | 0.25 |
| 10 | 175 nmol/L | 0.23 |
| 11 | 250 nmol/L | 0.13 |
| 12 | 250 nmol/L | 0.12 |

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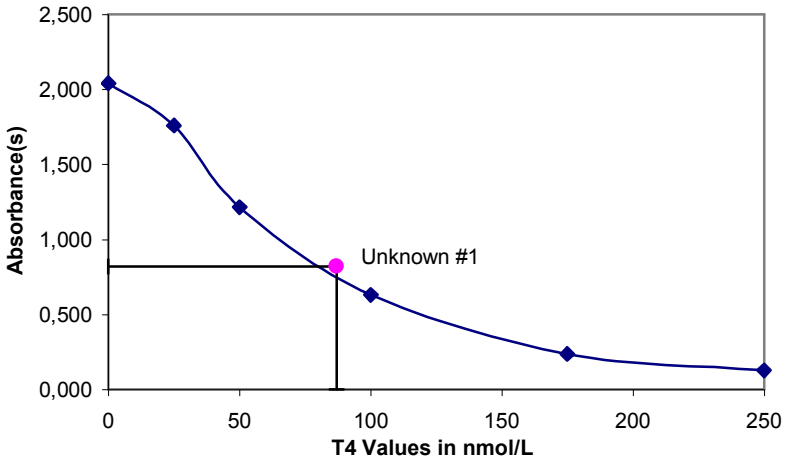
**Figure 1**




| | Unknown | | Avg. | |
|---|---|---|---|---|
| **Well** | **I. D.** | **O.D.** | **O.D.** | **Value** |
| 13 | Unknown #1 | 0,81 | | |
| 14 | Unknown #1 | 0,83 | 0,82 | 87 nmol/L |

*The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a standard curve prepared with each assay.

Assay Performance

Serum references and controls should not exhibit cloudiness with time. Discard if cloudiness is observed.
Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemeic or hemolysed specimen(s) should similarly not be used.
It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
Plate readers measure vertically. Do not touch the bottom of the wells.
Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
Unused microwell strips should be re-inserted into the aluminum foil bag and re-sealed with the ziploc.

## 9 REFERENCES / LITERATURE

1. Barker, S.B., "Determination of Protein Bound Iodine."
   Joumal Biological Chemistry, 173, 175, (1948).
2. Chopra, I.J., Solomon, D.H., an Ho, R.S., "A Radioimmunoassay of Thyroxine,"
   J. Clinical EndocrinoL, 33, 865 (1971).
3. Young, D.S., Pestaner, L.C., and Gilberman, U., "Effects of Drugs on Clinical Laboratory Tests."
   Clinical Chemistry, 21, 3660 (1975).
4. Sterling, L., Diagnosis and Treatment of Thyroid Disease, Cleveland
   CRC Press, P. 19 51 (1975).