Rodent T4

RODENT THYROXIN (T4) ELISA TEST KIT

LYOPHILIZED STANDARDS

The T4 ELISA test is an immunoassay designed for the quantitative determination of Thyroxin (T4) in serum/plasma samples of Rodent and related species.

TEST PRINCIPLE

In the T4 ELISA Test, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of unknown sample and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the T4 and conjugated T4 compete for the limited binding sites on the anti-T4 antibody. After 60 minutes of incubation period, at 37C, the wells are washed 5 times with wash buffer to remove any unbound T4 conjugate. A solution of TMB is then added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the absorbency is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T4 in the sample. A series of T4 standards assayed in the same way, a standard curve is constructed and the concentration of T4 in the unknown sample is quantified.

MATERIALS PROVIDED

1. Antibody-coated microtiter wells, 96-well plate
2. Lyophilized Standards (0, 5, 10, 25, 50, 100, 300ng/mL) reconstitute in 1mL standard/sample diluent.
3. HRP Conjugate Reagent, 12 mL
4. TMB Color Reagent (ready to use) 12 mL
5. Stop solution (2N HCl) 6mL
6. 20X Wash buffer, 20mL
7. Standard/Sample diluent, 20mL
8. Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED

1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Vortex mixer or equivalent
4. Absorbent paper or paper towel
5. Graph paper
6. Microtiter plate reader

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum/plasma samples only.

STORAGE OF TEST KIT AND INSTRUMENTATION

Note of Caution: Unopened test kits should be stored at 2-8^{O}C. The microtiter plate should always be kept in a sealed bag with desiccants to minimize exposure to damp air at room temperature. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. Do not leave any reagents at room temperature for more than 3 hours.

A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (25-28^{O}C) before use.
2. Lyophilized standards should be diluted in 1mL standard/sample diluent. This can be stored at -20*C, if not used immediately.
3. Prepare desired amount of wash buffer by diluting 1 part with 19 parts of distilled water. This buffer may be stored at 4-8C for 1-3 months.
4. Preparation of the samples: Mix 0.05mL of serum with 0.20mL of sample diluent and add 0.05mL per well. The samples diluted can be stored at –20C for further use. Dilution of sample will eliminate adding very low volumes (10ul) to the assay.

ASSAY PROCEDURE

One must follow accurately these steps to ensure correct results. Use clean pipettes and sterile, disposable tips:

1. Secure the desired number of coated wells in the holder.
2. Dispense 50ul of standards (ready to use) and add 50 ul diluted specimens into appropriate wells.
3. Dispense 100ul of T4 HRP-Conjugate Reagent into each well. Mix for 30 seconds. It is very important to mix well.
4. Incubate at 37^{O}C for 60 minutes.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times with wash buffer.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100ul of TMB color reagent into each well. Gently mix for 10 seconds.
9. Incubate at room temperature for 20 minutes, in the dark.
10. Stop reaction by adding 50ul of 2N HCl to each well.
11. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
12. Read optical density at 450nm with a microtiter well reader.

Important note: The wash step is very critical and insufficient washing will result in poor precision and falsely elevated absorbency readings.

CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/mL on graph paper, with absorbency values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of T4 in ng/mL from the standard curve.

EXPECTED VALUES AND SENSITIVITY

It is recommended that each laboratory should establish values to reflect differences specific to experimental conditions. The minimum detectable concentration of thyroxin by this assay is estimated to be 2ng/mL.

Limitations & Warranty

The present ELISA is designed for helping the scientist to analyze test samples only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test sample loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit damaged during shipment or leaking solutions within 30 days, with written explanation and return of the ELISA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples.

REFERENCES

1. Walker W.H.C. Introduction: An Approach to Immunoassay. **Clin. Chem.** 1977; 23: 384
2. Kirkegaard C., Friis T. and Siersback-Nielsen K. **Acta Endocrinol.** 1974; 77: 71
3. Wisdom G.B. Enzyme-Immunoassay. **Clin. Chem.** 1976; 22: 1243
4. Hoffenberg R. **Medicine** 1978; 8: 392
5. Lieblich J., Utiger R.D. **J. Clin. Invest.** 1972; 51: 1939

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QUALITY CONTROL DATA

NOTE: It is highly recommended that each laboratory must establish their own internal controls and normal reference values for desired age, sex and physiological parameters.

1. STANDARD CURVE

A typical T4 standard curve (illustration only) is given below. Please do not use these values to estimate or calculate your results.

Standard ng/ml	OD at 450nm
0	2.56
5	2.34
10	1.97
25	1.45
50	0.987
100	0.76
300	0.34

2. ASSAY PERFORMANCE

ELISA Performance Characters

Precision: Inter and Intra assay variation (CV) were determined from three different pooled serum samples in three different experiments.

Inter-assay variation	Set1: CV= 3.4%	Set2: CV= 5.2 %	Set3: CV= 5.7%
Intra-assay variation	Set1: CV= 7.6%	Set2: CV= 8.8 %	Set3: CV= 9.1 %
Number assayed	8	8	8

Sensitivity: The lowest level detectable in this assay is 2ng/ml of serum

Specificity: The T4 ELISA system utilizes monoclonal antibody and highly specific T4 HRP conjugate no cross-reactivity observed with T3 or related compounds