# RODENT TRIIODOTHYRONINE T3 ELISA TEST KIT
# LYOPHILIZED STANDARDS

The T3 ELISA test is an immunoassay designed for the quantitative determination of Triiodothyronine (T3) in serum/plasma samples of Rodent and related species.

## TEST PRINCIPLE

In the T3 ELISA Test, a mouse anti T3 antibody is coated on microtiter wells. A measured amount of Rodent serum/plasma and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During two-hour incubation at 37°C, the T3 and conjugated T3 compete for the limited binding sites on the anti-T3 antibody on the wells. After the incubation period, the wells are washed 5 times with wash buffer to remove any unbound T3 conjugate. A solution of TMB substrate 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 stop solution 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 T3 in the sample. By reference to a series of T3 standards assayed in the same way, the concentration of T3 in the unknown sample is quantified.

## MATERIALS PROVIDED

1. Antibody-coated 96-well plate
2. HRP Enzyme Conjugate, 12mL
3. Lyophilized Standards (0, 0.5, 1, 2.5, 5, 10, 20ng/mL) dilute in 1mL Standard/Sample Diluent
4. TMB Color Reagent (Ready to use) 12mL
5. Stop solution (2N HCl) 6mL
6. 20X Wash Buffer, 20 mL
7. Standard/Sample diluent, 20mL

## 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 of 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 rat and mouse serum/plasma samples only.

## STORAGE OF TEST KIT AND INSTRUMENTATION

**Note of Caution: Unopened test kits should be stored at 4-8$^{0}$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 including test samples should be brought to room temperature (18-25$^{0}$C) before use.
2. Must read and understand the instructions before attempting to use the kit.
3. Prepare Wash buffer by diluting 1 part with 19 parts of distilled water, excess amount may be stored at 4-8 C for couple of weeks.
4. Lyophilized standards should be diluted in 1ml using standard/sample diluent. This can be stored at -20C for long term use.

## ASSAY PROCEDURE

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

1. Secure the desired number of coated wells in the holder.
2. Dispense 50ul of standards, specimens, and controls into appropriate wells.

3. Dispense 100ul of Enzyme Conjugate Reagent into each well. Mix again. It is very important to mix well at this step.
4. Incubate at 37$^{o}$C for two hours.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times with washing buffer.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 100 ul of TMB solution 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 stop solution, 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 450 nm with a microtiter well reader.

*Important note:* The wash step is very critical. 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 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 T3 in ng/ml from the standard curve.

## EXPECTED VALUES AND SENSITIVITY

The minimal detectable concentration of T3 by this assay is estimated to be 0.2 ng/ml. and the normal and experimental values should be established in your own laboratory. Each laboratory must follow good laboratory practice and maintain proper documentation.

**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


Revised 02-21

Quality Control Data:
It is highly recommended that each laboratory must establish their own internal controls and normal reference values for desired pharmacological and physiological parameters.

A typical standard curve (illustration only) for rodent T3 is given below:

| **Standard ng/mL** | **OD at 450nm** |
|---|---|
| **20** | **0.520** |
| **10** | **0.661** |
| **5.0** | **0.968** |
| **2.5** | **1.350** |
| **1.0** | **1.934** |
| **0.5** | **2.212** |
| **0** | **2.88** |

**ELISA Performance Characters**

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

| | | | |
|---|---|---|---|
| Inter-assay variation | Set1: CV= 5.9% (N=10) | Set2: CV= 6.4 % (N=10) | Set3: CV= 4.4 % (N=10) |
| Intra-assay variation | Set1: CV= 8.9% (N=10) | Set2: CV= 5.4 % (N=10) | Set3: CV= 8.4 % (N=10) |

**Sensitivity**: The lowest level detectable in this assay is 0.2 ng/mL of serum or plasma

**Specificity**: The rodent T3 ELISA system utilizes monoclonal antibody to T3. The cross reactivity to other hormones including T4 is not detectable under the sensitivity of the assay system.