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# Mouse Rat Thyroxine (T4) Total ELISA Kit

## Catalog number: EK7013

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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# Mouse Rat Thyroxine (T4) Total ELISA Kit

**Catalog Number:** EK7013

**Size:** 96 wells/kit

**Sample Type:** Serum and plasma

**Sensitivity:** 1 μg/dl

**Assay Range:** 1-25 μg/dl

**Storage:** Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

## Introduction

This ELISA kit is of competitive format. Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest. The sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve. The standard curve is generated using the same principle but instead of adding samples, a series of recombinant molecules with known concentrations are added to 6-8 wells.

## Kit Components

| Description | Quantity |
|---|---|
| Microwells coated with T4 Monoclonal Ab | 12x8x1 Microwells |
| T4 Standards | 7 vials ( ready to use) 0.25 ml |
| T4 Enzyme (HRP) Conjugate concentrate | 1 vial 1.5ml |
| Assay Diluent | 1 bottle (ready to use) 12ml |
| TMB Substrate | 1 bottle (ready to use) 12ml |
| Stop Solution | 1 bottle (ready to use) 12ml |
| 20X Wash concentrate | 1 bottle 25ml |

### Standard Concentrations and example data

| | OD 450 nm | Conc. ng/mL |
|---|---|---|
| Std 1 | 2.615 | 0 |
| Std 2 | 1.982 | 1 |
| Std 3 | 1.627 | 2 |
| Std 4 | 1.075 | 5 |
| Std 5 | 0.646 | 10 |

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| | | |
|---|---|---|
| Std 6 | 0.471 | 15 |
| Std 7 | 0.325 | 25 |

## Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

## WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.

2. Potential biohazardous materials: The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.

3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. It is recommended that standards, control and serum samples be run in duplicate.

6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

7. Do not use **sodium azide** as preservative. Sodium azide inhibits HRP enzyme activities.

## SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.

2. Typically, specimens may be stored refrigerated at (2°C to 8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.

3. Avoid multiple freeze-thaw cycles.

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4. Prior to assay, frozen sera should be completely thawed and mixed well.

5. Do not use grossly lipemic specimens.

## REAGENT PREPARATION

T4-enzyme Conjugate Solution: Dilute the T4-enzyme conjugate 1:11 with assay diluent in a suitable container. For example, dilute 160µl of enzyme conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C).

General Formula:
Amount of Buffer required = Number of wells * 0.1
Quantity of Enzyme conjugate solution necessary = # of wells * 0.01
i.e. = 16 x 0.1 = 1.6ml for Total Conjugate Buffer
16 x 0.01 = 0.16ml (160µl) for enzyme conjugate solution.

Wash Buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

## ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 10 ul of the standards, control or specimen into the assigned well.

3. Add 100 ul of T4-enzyme conjugate solution to all wells (see Reagent Preparation Section).

4. Incubate for 60 minutes at room temperature with shaking.

5. Remove liquid from all wells. Wash wells three times with 300 ul of 1X wash buffer (see Reagent Preparation Section). Blot on absorbent paper towels.

6. Add 100 ul of TMB substrate solution to all wells.

7. Incubate, at room temperature, for fifteen (15) minutes.

8. Add 50 ul of stop solution to all wells and gently mix for 15-20 seconds.

9. Read the absorbance on ELISA Reader for each well at 450nm within 15 minutes after adding the stop solution.

## CALCULATION OF RESULTS

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The standard curve is constructed as follows:

1. Check T4 standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.

2. To construct the standard curve, plot the absorbance for T4 standards (vertical axis) versus T4 standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.