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**55R-FT4000**

## **Thyroxine ELISA Kit**

**Enzyme Immunoassay for the determination of Free Thyroxine (fT4) in human serum.**

**For Research Use Only, Not for Use in Diagnostic Procedures.**

## **1. Principle of the Test:**

The fT4 is a solid phase competitive ELISA. The unknowns, assay buffer and T4 enzyme conjugate are added to the wells coated with anti-T4 monoclonal antibody. fT4 in the serum competes with a T4 enzyme (HRP) conjugate for binding sites. Unbound fT4 and T4 enzyme conjugate is washed off by washing buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of fT4 in the unknowns. A calibration curve is prepared relating color intensity to the concentration of fT4.

## **2. Warnings and Precautions:**

### **1. 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.

2. This kit is designed for research use only, not for use in diagnostic procedures.

3. Not for internal or external use in humans or animals.

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

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

6. It is recommended that calibrators, control and serum unknowns be run in duplicate.

7. 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.

## **3. Storage and Stability:**

1. Store the kit at 2 – 8° C.

2. Keep Microwell sealed in a dry bag with desiccants.

3. The reagents are stable until expiration of the kit.

4. Do not expose test reagents to heat, sun, or strong light.

#### **4. Contents of the Kit:**

1. Microwells coated with fT4 MAb 12x8x1
2. fT4 Calibrator: 6 vials (ready to use) 0.5ml
3. fT4 enzyme conjugate: 1 Bottle (ready to use) 12 ml
4. TMB Substrate: 1 bottle (ready to use) 12ml
5. Stop Solution: 1 bottle (ready to use) 12ml
6. 20X Wash concentrate: 1 bottle 25ml

#### **Additional materials and equipment required but not provided in the kit:**

1. Distilled or de ionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

#### **5. Collection and Handling of Unknowns:**

1. Collect blood and separate the serum immediately.
2. Unknowns may be stored refrigerated at (2-8° 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.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic unknowns.

## **6. Reagent Preparation:**

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 (18-26°C).

## **7. Test Procedure:**

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C).

1. Format the microplate wells for control, calibrators and unknowns to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 50 µl of fT4 calibrators, control and unknowns into the assigned well.
3. Add 100 µl of fT4 enzyme conjugate to all wells.
4. Incubate for 60 minutes at room temperature (18-26° C).
5. Remove liquid from all wells. Fill wells with 300 µl 1X wash buffer (see buffer preparation above). Wash three times. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

## 8. Results:

1. The calibration curve is constructed as follows:
2. Check fT4 calibrator value on each calibrator vial. This value might vary from lot to lot. Make sure you check the value on every kit.

Example calibrator values:

Calibrator 1	0 ng/dL
Calibrator 2	0.45 ng/dL
Calibrator 3	1.10 ng/dL
Calibrator 4	2.10 ng/dL
Calibrator 5	5.00 ng/dL
Calibrator 6	7.70 ng/dL

3. To construct the calibration curve, plot the absorbance for fT4 calibrators (vertical axis) versus fT4 calibrator concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
4. Read the absorbance for controls and each unknown from the curve. Record the value for each control or unknown.

## 9. Limitations of the Test:

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

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