



Thyroxine (T4) (Mouse/Rat) ELISA Kit

6/14

(Catalog # K7421-100, 100 assays; Store at 2-8°C)

I. Introduction:

T4 is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T4 is decreased in hypothyroid patients and is increased in hyperthyroid patients. BioVision's mouse/rat thyroxine (T4) kit is a solid phase competitive ELISA Kit. The samples, and T4 enzyme conjugate are added to the wells coated with anti-T4 polyclonal antibody. T4 in the sample competes with a T4 enzyme (HRP) conjugate for binding sites. Unbound T4 and T4 enzyme conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of T4 in the samples. A standard curve is prepared relating color intensity to the concentration of the T4.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Mouse/rat thyroxine.

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K7421-100	Part No.
Plate coated with T4 Ab	12 stripsx8 wells	K7421-100-1
T4 Standard: (0.25 ml)	7 vials	K7421-100-2.x
Assay Diluent	12 ml	K7421-100-3
T4 Enzyme Conjugate Conc.	1.5 ml	K7421-100-4
Wash Concentrate (20X)	25 ml	K7421-100-5
TMB Substrate	12 ml	K7421-100-6
Stop Solution	12 ml	K7421-100-7

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Concentrate:** 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).
- **T4-Enzyme Conjugate Solution:** Dilute the T4-enzyme conjugate 1:11 with assay diluent in a suitable container. For example, dilute 160 µl of enzyme conjugate with 1.6 ml of diluent for 16 wells (A slight excess of solution is made). This reagent should be used within 24 hrs for maximum performance of the assay. Store at 2-8°C.

VIII. Warning & Precautions:

- Potential biohazardous materials: The calibrator & controls contains animal and 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.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- 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.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. Specimens 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. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

X. Assay Protocol:

Prior to assay, bring reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use. 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.

1. Format the microplate wells for each serum reference, control, and sample to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipet 10 µl of appropriate serum reference, control, and samples into designated wells.
3. Add 100 µl of diluted T4 enzyme conjugate to all wells. Shake gently for 20-30 sec. to mix.
4. Cover the plate and incubate for 60 min. at room temperature (18-26°C) with shaking.
5. Remove liquid from all wells & wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells & incubate for 15 min. at room temperature.



7. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
8. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stopping solution.

XI. Calculation: Construct the standard curve; plot the absorbance for the T4 standards (vertical axis) versus the T4 standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve:

Standard	OD (450 nm)	Part No.
Standard 1 (0 µg/dl)	2.615	K7421-100-2.1
Standard 2 (1 µg/dl)	1.982	K7421-100-2.2
Standard 3 (2 µg/dl)	1.627	K7421-100-2.3
Standard 4 (5 µg/dl)	1.075	K7421-100-2.4
Standard 5 (10 µg/dl)	0.646	K7421-100-2.5
Standard 6 (15 µg/dl)	0.471	K7421-100-2.6
Standard 7 (25 µg/dl)	0.325	K7421-100-2.7

Sensitivity: The Thyroxine test system procedure has a sensitivity of 0.467 µg/dl. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 20(95%certainty) statistics to calculate the minimum dose.

Expected Values: Using BioVision T4 mouse/rat ELISA kit, the normal serum/plasma sample is expected to contain between 2.37 µg/dl – 9.70 µg/dl T4. It is recommended that each laboratory establish its own range of expected values for the population being tested.

XII. RELATED PRODUCTS:

Human CellExp™ TPO, human recombinant (6483)
Thyroid Stimulating Hormone (human) ELISA Kit (K7411)

TPO (mouse) ELISA Kit (4753)
Thyroxine (T4) (human) ELISA kit (K7413)

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