



Thyroxine (T4) (Human) ELISA Kit

(Catalog # K7413-100, 100 assays; Store at 2-8°C)

rev 06/20

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 human thyroxine (T4) kit is a solid phase competitive ELISA Kit. The samples, working T4-HRP Conjugate and Anti-T4-Biotin Solution are added to the wells coated with Streptavidin. T4 in the sample competes with a T4 enzyme (HRP) conjugate for binding sites. Unbound T4 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 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:

Human Thyroxine.

IV. Sample Type:

- Serum or plasma

V. Kit Contents:

Components	K7413-100	Part No.
Microplate coated with Streptavidin	12 stripsx8 wells	K7413-100-1
T4 Standard: (0.5 ml) (ready to use)	6 vials	K7413-100-2
Anti-T4-Biotin Solution	7 ml	K7413-100-3
T4 -HRP Conjugate (11X)	0.7 ml	K7413-100-4
T4 Assay Diluent	7 ml	K7413-100-5
TMB Substrate (ready to use)	12 ml	K7413-100-6
Stop Solution (ready to use)	12 ml	K7413-100-7
Wash Concentrate (20X)	25 ml	K7413-100-8

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 conjugate buffer in a suitable container. For example, dilute 80 µl of enzyme conjugate with 0.8 ml of buffer 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 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:

Note: 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 25µl of the standards, control or specimen into the assigned well.
3. Add 50µl of the working T4-enzyme conjugate solution to all wells
4. Add 50µl of T4-Antibody-Biotin Solution to all wells.
5. Swirl the microplate gently for 20-30 seconds to mix the reagents.
6. Cover and Incubate 60 minutes at room temperature.
7. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.



8. Add 100µl of TMB substrate solution to all wells
9. Cover the plate and incubate at room temperature for fifteen (15) minutes.
10. Add 50µl of stop solution to each well and gently mix for 15-20 seconds.
11. Read the absorbance on ELISA Reader for each well at 450nm within 15 minutes after adding the stop 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)
Standard 1 (0 µg/dL)	2.18
Standard 2 (2 µg/dL)	1.50
Standard 3 (5 µg/dL)	1.19
Standard 4 (10 µg/dL)	0.86
Standard 5 (15 µg/dL)	0.65
Standard 6 (25 µg/dL)	0.44

Expected Values: A study of euthyroid adult population was undertaken to determine expected values for the T4 EIA test system. The mean value, standard deviation and expected ranges of samples are presented in the following table:

	Male (42 samples)	Female (58 samples)
Mean	7.6	8.2
Standard Deviation	1.6	1.7
Expected range	4.4-10.8	4.8-11.6

XII. RELATED PRODUCTS:

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

TPO (mouse) ELISA Kit (4753)