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Triiodothyronine (T3) (Human) ELISA Kit

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(Catalog # K7423-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

Triiodothyronine (T3) is a hormone produced by the thyroid gland. T3 plays an important role in a majority of the physiological processes in the body such as growth, development, metabolism, heart rate, and body temperature. The hormone exists in 2 forms: almost all of T3 is mainly bound to plasma proteins namely Thyroxine-binding globulin and serum albumin. The free unbound T3 is the biologically active form of the hormone. The anterior pituitary gland produces the Thyroid-stimulating hormone which further stimulates the production of T3, along with thyroxine (T4). The 3 hormones are engaged in a closed-loop feedback process; as the concentrations of T3 and T4 increase in the blood plasma, the anterior pituitary stops the production of TSH; when T3 and T4 concentrations decrease, TSH production increases. If an excess amount of T3 and T4 is produced by the thyroid gland, it results in hyperthyroidism whereas insufficient amounts lead to hypothyroidism. **BioVision's Triiodothyronine (T3) (Human) ELISA Kit** is designed to measure the amount of total Triiodothyronine (bound as well as unbound forms of T3) in human serum samples and to evaluate the functioning of the thyroid gland. The kit is based on the Competitive ELISA principle. Samples, Standards, Assay diluent, and T3 enzyme conjugate are added to the wells coated with an anti-T3 monoclonal antibody. The T3 present in the samples will compete with the T3 enzyme conjugate for binding to the antibody. Any unbound T3 from the serum and enzyme conjugate is washed off with the wash buffer. The addition of the substrate results in color development that is inversely correlated to the concentration of the T3 in the samples.

II. Application:

Quantitative detection of total Triiodothyronine (T3) in human serum sample

III. Specificity:

Human Triiodothyronine (T3)

IV. Sample Type:

Serum

V. Kit Contents:

Components	K7423-100	Part No.
Plate coated with T3 MAb	8 x 12 strips	K7423-100-1
T3 Standards	0.5 ml X 6	K7423-100-2.x
T3 Assay Diluent	12 ml	K7423-100-3
T3 Enzyme Conjugate Conc. (11X)	1.2 ml	K7423-100-4
Wash Concentrate (20X)	25 ml	K7423-100-5
TMB Substrate	12 ml	K7423-100-6
Stop Solution	12 ml	K7423-100-7

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^{o}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 Buffer (1X):** Prepare **Wash buffer (1X)** by adding the contents of the bottle (25 ml, **Wash Concentrate 20X**) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).
- **T3 Enzyme Conjugate solution:** Dilute the **T3 Enzyme Conjugate Conc. (11X)** in the ratio 1:11 with **T3 Assay diluent**. For e.g. dilute 160 µl of **T3 Enzyme Conjugate Conc. (11X)** with 1.6 ml **T3 Assay diluent** for 16 wells. This working solution must be used within 24 hours to ensure maximum performance of the assay. Store the solution at 2-8°C. Use the general formula to calculate the amount of enzyme conjugate to prepare:
  Amount of T3 Assay diluent required = Number of wells x 0.1
  Quantity of T3 enzyme necessary = Number of wells x 0.01
  Hence, 16 wells x 0.1 = 1.6 ml T3 Assay diluent and 16 wells x 0.01 = 0.16 ml (160 µl) T3 enzyme conjugate

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:

Prior to assay, bring reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use. Check T3 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 microplates' wells for control, standard and patient samples to be assayed in duplicate. Place any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 50 µl of **T3 standards**, **control** and **samples** into the assigned well.

3. Add 100 µl of **T3 enzyme conjugate solution** to all wells.
4. Mix the microplate well for 20 – 30 secs and then incubate for 60 minutes at room temperature (18-26°C).
5. Remove liquid from all wells. Fill wells with 300 µl **Wash buffer (1X).** 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.

XI. Calculation:

The standard curve is constructed as follows:

1. Check T3 standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit.
2. To construct the standard curve, plot the absorbance for T3 standards (vertical axis) versus T3 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.

Example of a Standard Curve: The OD values for the standard curve are for demonstration purposes only.

Standard	OD (450 nm)
Standard 1 (0 ng/ml)	2.568
Standard 2 (0.5 ng/ml)	1.706
Standard 3 (1.5 ng/ml)	1.223
Standard 4 (3.0 ng/ml)	0.611
Standard 5 (6 ng/ml)	0.330
Standard 6 (9 ng/ml)	0.186

Assay Sensitivity: Sensitivity of the ELISA was determined by running 24 replicates of the Zero Standard in the assay and the +2SD from the mean of off the dose response curve was replotted

Standard	Mean + 2SD (Sensitivity)
Zero standard	0.066 ng/ml

Assay Precision:

1. **Intra-assay precision** was calculated by assaying 16 replicates of each of the 3 sera: normal, low and high

Serum	No of replicates	Mean (ng/ml)	SD	Coefficient of Variation (%)
1	16	1.25	0.09	6.9%
2	16	2.41	0.09	3.7%
3	16	4.12	0.13	3.2%

2. **Inter-assay precision** was calculated by assaying duplicates of 3 serum pools in 16 separate runs. A standard curve was constructed for each run

Serum	No of replicates	Mean (ng/ml)	SD	Coefficient of Variation (%)
1	16	1.2	0.109	8.91%
2	16	2.5	0.146	5.89%
3	16	4.1	0.194	4.72%

Assay Specificity: Interfering substances of various concentrations were added to the serum matrix to check for cross-reaction of the Thyroxine antibody to these substances. A ratio of dose of interfering substance to dose of Thyroxine needed to displace the same amount of tracer was used to calculate the cross-reactivity.

Analyte	Highest concentration tested	Cross-reaction percent (%)
T3	-	100%
T2	156.25	4.29%
T4	625	0.81%
Sodium Salicylate	625	0.06%
Phenyl Butazone	625	0.07%

Limitations of the Test

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

XI. Related Products:

Human CellExp™ TPO, human recombinant (6483)
Thyroid Stimulating Hormone (human) ELISA Kit (K7411)
Triiodothyronine (T3) (Mouse/Rat) ELISA kit (K7422)
Thyroid Stimulating Hormone (human) ELISA Kit (K7411)
Thyroxine (T4) (Mouse/Rat) ELISA kit (K7421)
Thyroxine (T4) (human) ELISA kit (K7413)

FOR RESEARCH USE ONLY! Not to be used on humans.