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User's Manual

Human Total T4 (Thyroxine) ELISA Kit

REF **IVDIA1011-FA**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Human Total T4 (Thyroxine) ELISA Kit is a quantitative solid phase enzyme linked immunsorbent assay. This test provides quantitative measurement of Total T4 (THYROXINE) in human serum to aid in the diagnosis of disease.

General Description

The thyroid gland produces thyroxine T4, triiodothronine T3 and calcitonin. The first two hormones are synthesized by the gland following entrapment of iodide, conversion to iodine, and coupling of iodine with tyrosine, followed by coupling of two iodinated tyrosine molecules. T4 and T3 so formed are attached to thyroglobulin for storage and are released, as needed, as protease splits them from globulin.

Thyroxine is a highly active thyrometabolic hormone that exists in protein-bound and unbound forms. For T4 can be measured more easily and with greater accuracy than T3, determination of total T4 by immunoassay is the most reliable for detecting thyroid disorders in man.

Release of T4 and T3 from the thyroid is greatly influenced by pituitary-thyroid stimulating hormone (TSH) that in turn is influenced by hypothalamic thyrotropin-releasing hormone (TRH). Normally, increased blood levels of T4 and T3 act to decrease the amount of TSH secreted, thereby reducing the production and release of T4 and T3. Decreaded blood levels of T4 and T3 produce the opposite effect, leading to increased production and secretion of T4 and T3.

In this manner a normal circulating thyroid hormone balance is maintained. Circulating T4 and T3 are bound largely to thyroxine binding globulin (TBG). To a lesser extent they are bound to thyroxine binding prealbumin (TBPA) and, when present in excess, to albumin. Usually T4 to T3 concentration ratio is about 9:1, However T3 has considerably greater physiological activity. It is the small free fraction (0.1% of the total or less) that is physiologically active and determines the clinical thyroid status of the patient's hyperthyroid, euthyroid, or hypothyroid.

Principles of Testing

Human Total T4 (Thyroxine) ELISA Kit Quantitative method is based on the principle of competitive solid phase enzyme immunoassay. Test specimen and enzyme labeled T4 are incubated in an antibody coated microwell. The T4 in the specimen competes with the labeled T4 for a limited number of binding sites on the well.

In performing the assay procedures, T4 standard or patient's serum and T4 horseradish peroxidase conjugate are added to interact with T4 antibody coated on the well. In this solid-phase system the antibody-bound T4 remains on the surface of the well whereas free T4 are removed by decantation or aspiration of the liquid phase Antibody bound T4-horseradish peroxidase conjugate is then allowed to react with TMB reagent, and a color is developed after a short incubation period. The enzyme reaction is stopped and the intensity of the color measured with micro-well reader at 450 nm. When high levels of patient T4 are present in patient's serum, less enzyme conjugate is bound. Hence less color development is observed.

Reagents And Materials Provided

1. Microwell Strips: Anti-T4 antibodies coated wells, 8 x 12 strips, 96 wells
2. Enzyme Conjugate (11 mL): T4 conjugated to horseradish peroxidase
3. TMB Solution (11 mL): containing H2O2 and TMB
4. Reference Standard Set (0.6.0 mL each vial): Calibrated to 0, 1.5, 3.0, 6.0, 12 and 24 µg/dL in the human serum base.
5. Low and High Control(0.6.0 mL each)
6. Stop Solution: 2 N HCl.
7. Washing Buffer Concentrate (100X) (10 mL): Prepare working washing solution by adding 10 mL washing buffer concentrate into 990 mL distilled water.
8. Well Holder: For securing individual wells.

Materials Required But Not Supplied

1. Microwell Reader
2. Pipetor with tips for 25 and 100 µL
3. 1 L washing bottle.

Storage

1. Store the kit at 2-8°C in a refrigerator.
2. Keep microwells sealed in a dry bag with desiccants.
3. The unopened reagents are stable until expiration of the kit. TMB Solution should be colorless; if solutions turn blue, it must be replaced. Do not exp

Specimen Collection And Preparation

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation at room temperature. If sera cannot be assayed immediately, they can be stored at 2-8°C for a week or frozen for up to six months. Avoid repeated freezing and thawing of serum sample. The use of hemolyzed or lipemic samples are not recommended.

Assay Procedure

Preparation for Assay

1. Before beginning the test, bring all samples and reagents to room temperature (24±3°C) and gently mix.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruptions to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µL of Standards, Controls or Serum samples.

3. Dispense 100 µL of enzyme conjugate to each wells
4. Incubate for 60 minutes at room temperature.
5. Remove incubation mixture and rinse the wells 5 times with washing buffer solution (300 µL/well/each rinse).
6. Dispense 100 µL of TMB Solution into each well.
7. Incubate 30 minutes at room temperature.
8. Stop reaction by adding 50 µL of stop solution into each well.
9. Read O.D. at 450 nm with a microwell reader against the blank well that contains only TMB Solution and Stop Solution.

Quality Control

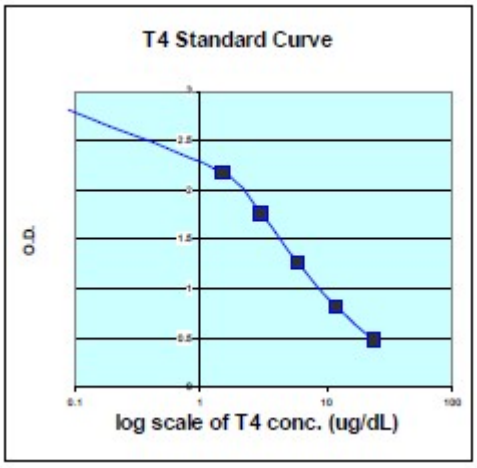
Each laboratory should utilize internal controls at several levels to monitor assay performance. The control should be treated as unknown. Results obtained should be in agreement with the assigned value of the control. The controls can be obtained from commercially available sources, but should not contain sodium azide as preservatives.

Calculation

Any microwell reader capable of determining at 450 nm may be used. The T4 value of patient is obtained as follows:

1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on graph paper.
2. Obtain the value of patient T4 by reference to the Standard Curve. For example: (This data is for demonstration purposes only and must not be used in place of data generated for each assay).

Well No.	Description (ug/dL)	Absorbance 450 nm	T4 (ug/dL)
A1	0.00	2.859	
B1	0.00	2.803	
A2	1.50	2.174	
B2	1.50	2.187	
A3	3.0	1.743	
B3	3.0	1.896	
A4	6.0	1.283	
B4	6.0	1.283	
A5	12.0	0.792	
B5	12.0	0.748	
A6	24.0	0.478	
B6	24.0	0.482	
A7	Patient I	1.695	3.3
B7	Patient II	0.880	10.7



Reference Values

Use of T4 ELISA reagent in a study of 75 euthyroid patients in one geographic location will yield a normal range (4-12 µg/dL) at the 95% confidence limit. It is recommended that laboratories adjust normal values to reflect geographic and population differ

Precision

Intra-assay Precision: 8.23%-8.39%

Inter-assay Precision: 9.33%-11.29%

Recovery

Equal volume of known (3 µg/dL and 6 µg/dL) sample T4 was added to equal volume of various known concentrations of T4. Samples were tested and T4 recoveries were compared with the expected concentrations.

A serum containing 24 µg/dL T4 was diluted in T4 free serum. The dilutions were tested and the T4 recoveries were compared with the expected concentrations.

Precautions

1. It is designed for in vitro use only.
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Limitations

1. Extrapolation of T4 values beyond the standard curve may yield variable results. Sample containing 24 µ g/dL can be diluted with the T4 free human serum and retested.
2. Samples containing T4 less than 1.5 µg/dL are analyzing by diluting the 1.5 µg/dL T

References

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2. Oppenheimer, J.H. Role of Plasma Proteins in the binding, distribution and metabolism of the thyroid hormones. New Engl. J. Med. 278:1153-1162.
3. Schall R., Fraser, A., Hansen