ab108661

Thyroxine (T4) Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Thyroxine (T4) concentrations in serum

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

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1. Introduction

ab108661, Thyroxine (T4) Human ELISA Kit is intended for the quantitative determination of Total Thyroxine (T4) concentration in Human serum.

L-Thyroxine (T4) is a hormone that is synthesized and stored in the thyroid gland. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of Thyroxine (T4) is reversibly bound to three plasma proteins in blood - thyroxine binding globulin (TBG) binds 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%. Approximately 0.03% of T4 is in the free, unbound state in blood at any one time.

2. Assay Summary

ab108661 measures Thyroxine (T4) by competitive immunoassay techniques. A sample of serum or plasma containing the T4 to be quantified is mixed with labeled T4 and T4 antibody The labeled T4 contains 8-anilino-1-napthalene sulfonic acid (ANS) to inhibit binding of T4 to serum proteins. A fixed amount of labeled T4 competes with the unlabeled T4 in the sample for a fixed number of binding sites on the specific T4 antibody.

Antibody to Thyroxine (T4) is coated on a solid phase (microtiter well). A measured amount of serum and a constant amount of Thyroxine (T4) labeled with horseradish peroxidase are added. During incubation, T4 in the sample and enzyme-labeled T4 compete for the limited binding sites on the T4 antibody.

After a 60 minute incubation at room temperature, the wells are washed 5 times by water to remove unbound Thyroxine (T4) conjugate.

A solution of tetramethylbenzidine (TMB) reagent is added and incubated for 20 minutes, resulting in the development of a blue color.

The color development is stopped with the addition of Stop Solution. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled Thyroxine (T4) in the sample. Absorbance is measured spectrophotometrically at 450 nm.

3. Kit Contents

- Antibody-Coated Wells (1 plate, 96 wells); microtiter wells coated with Sheep anti-Thyroxine (T4).
- Reference Standard Set (1 set, 1.0 ml/vial); contains 0, 2, 5, 10, 15, and 25 µg/dl, ready to use.
- Enzyme Conjugate Concentrate (11x) 1.3 ml Thyroxine (T4) antigen conjugated to horseradish peroxidase.
- Enzyme Conjugate Diluent (13 ml) contains 8-anilino-1-napthalene sulfonic acid (ANS).
- TMB Reagent (11 ml).
- Stop Solution (11 ml).

4. Storage and Handling

Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.

5. Additional Materials Required

- Distilled or deionized water
- Precision pipettes: 25 µl, 100 µl and 1.0 ml
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450 nm. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.
- Vortex mixer, or equivalent
- Absorbent paper
- Graph paper

6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. To prepare **Working Conjugate Reagent**, add 0.1 ml of Enzyme Conjugate Concentrate (11x) to 1.0 ml of Thyroxine (T4) Conjugate Diluent (1:10 dilution), and mix well.

   Note: Prepare only the amount of Conjugate that is required each time. Working Conjugate Reagent should be used within 24 hours. Discard the excess after use.

7. Preparation and Collection of Specimen

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

8. Assay Method

Assay Procedure:

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Working Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.

10. Incubate in the dark at room temperature for 20 minutes.

11. Stop the reaction by adding 100 µl of Stop Solution to each well.

12. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***

13. Read absorbance at 450nm with a microtiter well reader ***within 15 minutes.***

9. Data Analysis

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µg/dl on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

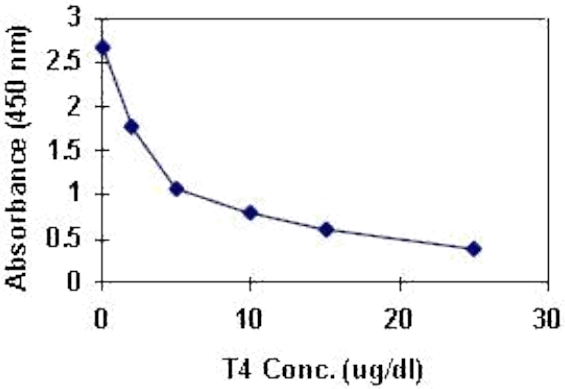
3. Using the mean absorbance value for each sample, determine the corresponding concentration of Thyroxine (T4) in µg/dl from the standard curve.

A. Typical Data

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against Thyroid Thyroxine (T4) concentrations shown on the X axis.

NOTE: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

Thyroxine (T4) (µg/dl)	**Absorbance (450 nm)**
0	2.667
2	1,786
5	1.060
10	0.778
15	0.591
25	0.384



B. Sensitivity

The minimum detectable concentration of Thyroxine (T4) by this assay is estimated to be 0.4 µg/dl.

10. Limitations

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

11. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4 °C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution

Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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