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ab108685 – Triiodothyronine (T3) ELISA Kit

Instructions for Use

A competitive immunoenzymatic assay for the quantitative measurement of Triiodothyronine (T3) in serum and plasma.

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

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Table of Contents

INTRODUCTION

1. BACKGROUND 2
2. ASSAY SUMMARY 4
3. PRECAUTIONS 5

GENERAL INFORMATION

4. STORAGE AND STABILITY 5
5. MATERIALS SUPPLIED 5
6. MATERIALS REQUIRED, NOT SUPPLIED 6
7. LIMITATIONS 6
8. TECHNICAL HINTS 7

ASSAY PREPARATION

9. SAMPLE COLLECTION AND STORAGE 8
10. PLATE PREPARATION 9

ASSAY PROCEDURE

11. ASSAY PROCEDURE 10

DATA ANALYSIS

12. CALCULATIONS 12
13. TYPICAL DATA 13
14. TYPICAL SAMPLE VALUES 14
15. ASSAY SPECIFICITY 15

RESOURCES

16. TROUBLESHOOTING 16
17. NOTES 18

1. BACKGROUND

Abcam's Triiodothyronine (T3) *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Triiodothyronine in serum and plasma.

A 96-well plate has been precoated with anti-T3. Samples and the T3-HRP conjugate are added to the wells, where Triiodothyronine in the sample competes with the added T3-HRP for antibody binding. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Triiodothyronine in the sample and the intensity is measured at 450 nm.

Triiodothyronine (T3) is a tyrosine-based hormone produced by the thyroid gland. Iodine is an important component for T3 synthesis.

Thyroxine-binding globulin (TGB) is the major carrier protein for circulating thyroid hormone. Only the free fraction of T3 is biologically active, a very small fraction of the circulating hormone is unbound - T3 0.3%.

Thyronines act on the body to increase the basal metabolic rate, affect protein synthesis and increase the body's sensitivity to catecholamines (such as adrenaline). The thyroid hormones are essential for proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis.

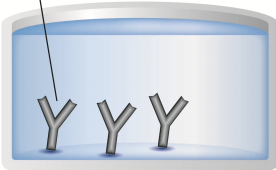
Both excess and deficiency of thyroxine can cause disorders. Thyrotoxicosis or hyperthyroidism is the clinical syndrome caused by an excess of circulating free thyroxine, free triiodothyronine, or both. It is a common disorder that affects approximately 2% of women and 0.2% of men. Hypothyroidism is caused when there is a deficiency of thyroxine.

Every day about 5-8 micrograms of T3 is released into the blood from the thyroid gland. In addition, approximately 22 mg/day of T3 is produced by nonthyroidal 5-monodeionination of T4. T3 has a much faster turnover than T4 (T3 ~1 day, compared to ~6 days for T4) and has greater biological potency than T4.

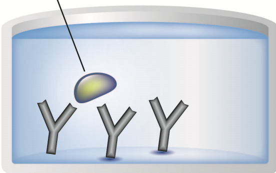
Several drugs are known to affect the binding of Triiodothyronine to the thyroid hormone carrier proteins and its metabolism to T3. These drugs complicate the interpretation of T3 results. Circulating auto antibodies to T3 and hormone-binding inhibitors may also interfere.

Measurement of total serum T3 concentrations is a standard and well-validated test of thyroid gland function

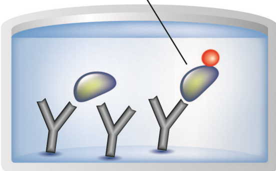
2. ASSAY SUMMARY



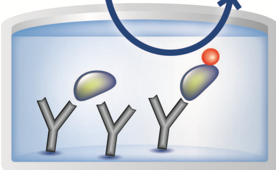
Prepare all reagents, samples, controls and standards as instructed.



Add samples, standards and controls to wells used.



Add prepared labeled HRP-Conjugate to each well. Incubate at room temperature 22-28ºC.



After washing, add TMB substrate solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-T3 IgG Coated Microplate (12 x 8 wells)	96 Wells	2-8°C
Stop Solution	15 mL	2-8°C
11X T3-HRP Conjugate	1.4 mL	2-8°C
TMB Substrate Solution	15 mL	2-8°C
Conjugate Buffer	12.5 mL	2-8°C
50X Wash Solution	20 mL	2-8°C
T3 Standard 0– 0.0 ng/mL	1 mL	2-8°C
T3 Standard 1 – 0.5 ng/mL	1 mL	2-8°C
T3 Standard 2 – 1.0 ng/mL	1 mL	2-8°C
T3 Standard 3 – 2.5 ng/mL	1 mL	2-8°C
T3 Standard 4 – 5.0 ng/mL	1 mL	2-8°C
T3 Standard 5 – 7.5 ng/mL	1 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm or 620 nm
- Multi- and single-channel pipettes to deliver volumes between 10 and 1,000 µL
- Optional: Automatic plate washer for rinsing wells.
- Rotating mixer
- Deionised or (freshly) distilled water.
- Disposable tubes
- Timer

7. LIMITATIONS

- ELISA kit intended for research use only. Not for use in diagnostic procedures
- All components of Human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious
- Use only clean pipette tips, dispensers, and lab ware
- Do not interchange screw caps of reagent vials to avoid cross-contamination
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use

- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate, without splashing, accurately to the bottom of wells

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents, samples and controls to room temperature (18-25°C) prior to use for at least 30 minutes. At the end of the assay return the reagents to 2-8°C, avoid long exposure to room temperature.

9.1 **1X Wash Solution**

Dilute concentrated Wash Solution to 1000 ml with distilled or deionised water in a suitable storage container. For smaller volumes respect the 1:50 ratio. The diluted wash solution is stable for 30 days at 2-8°C.

9.2 **1X T3-HRP Conjugate**

Dilute the T3-HRP conjugate 1:11 with Conjugate Buffer in a suitable container. This reagent should be used within 24 hours for maximum performance of the assay.

Store at 2-8°C

10. SAMPLE COLLECTION AND STORAGE

Collect samples by venipuncture in 10 mL silicone evacuated tubes. The usual precautions in the collection of venipuncture samples should be observed. Separate the red blood cells by centrifugation use serum for the T3 procedure. Specimens may be refrigerated at 2÷8°C (for a maximum period of 48 hours). If the specimens cannot be assayed within 48 hours, the samples may be stored at temperatures of –20°C for up to 30 days. When assayed in duplicate, 0.10mL of the specimen is required.

Avoid repeated freezing and thawing

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 1 well must be used as a blank, omitting sample and conjugate from well addition
- For statistical reasons, we recommend each standard and sample should be assayed with a minimum of two replicates (duplicates)

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described.**
- **If performing the test on ELISA automatic systems we recommend increasing the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects.**
- **Assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3. Add 50 µL standards, and samples into their respective wells. Add 100 µL diluted T3-HRP Conjugate to each well. Leave a blank well for substrate blank.

13.4. Cover wells with the foil supplied in the kit and incubate for 1 hour at room temperature (22-28°C).

13.5. Remove the foil, aspirate the contents of the wells and wash each well three times with 300 µL of 1X Washing Solution. Avoid spill over into neighboring wells. The soak time between each wash cycle should be >5 sec. After the last wash, remove the remaining 1X Washing Solution by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid. (if you use automated equipment, wash the wells at least 5 times)
Important note: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

Note: Complete removal of liquid at each step is essential for good assay performance.

13.6. Add 100 µL TMB Substrate Solution into all wells.

13.7. Incubate for exactly 15 minutes at room temperature (22-28°C) in the dark.

13.8. Add 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently. Any blue color developed during the incubation turns into yellow.

13.9. Measure the absorbance of the sample at 450 nm against a reference wavelength of 620-630 nm or against the Blank within 5 minutes.

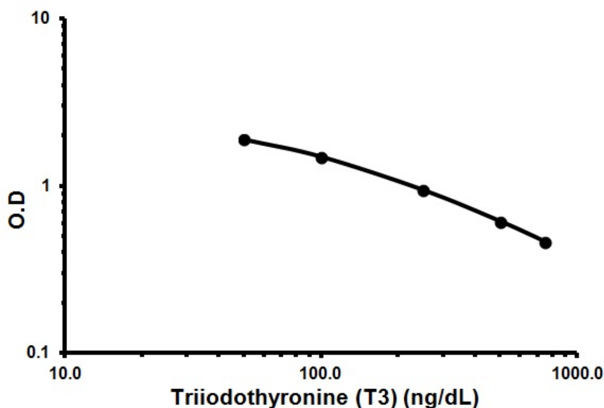
13. CALCULATIONS

Calculate the mean background subtracted absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (e. g.: Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/dL.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



Conc. (ng/dL)	O.D
0	2.36
50	1.90
100	1.50
250	0.95
500	0.61
750	0.46

15. TYPICAL SAMPLE VALUES

REFERENCE VALUES-

A study of euthyroid adult population was undertaken to determine expected values for the T3 EIA Test System:

Mean (ng/mL)	SD	Range (ng/mL)
1.185	0.334	0.52 – 1.85

SENSITIVITY –

The lowest detectable concentration of T3 that can be distinguished from the zero standard is 0.05 ng/mL at the 95 % confidence limit.

PRECISION –

	Intra-Assay	**Inter-Assay**
n=	40	3
%CV	≤ 10.7	≤ 9.1

RECOVERY –

The recovery of 0.5, 1.0, 2.0 and 4 ng/mL of T3 added to samples gave an average value (±SD) of 97.5% ± 4.0 % with reference to the original concentrations.

16. ASSAY SPECIFICITY

The cross reactivity of the T3 antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of T3 needed to displace the same amount of tracer.

Substance	Concentration	Cross Reactivity
l-Triiodo-thyronine	-	1,0000
l-Thyroxine	10 µg/dL	0,0100
d-Thyroxine	10 µg/dL	0,0025
d-Triiodo-thyronine	100 µg/dL	0,0150
Monoiodo Tyrosine	100 µg/mL	N/D
Diiodo-Tyrosine	100 µg/mL	N/D
Triiodothyroacetic Acid	100 µg/mL	N/D
Tetraiodothyroacetic Acid	100 µg/mL	N/D

17. TROUBLESHOOTING

Problem	Cause	Solution
Low signal	Incubation time to short	Try overnight incubation at 4 °C
	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
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 & ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (e.g. minimize freeze/thaws cycles)

Problem	Cause	Solution
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
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

18. NOTES

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Technical Support

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