Triiodothyronine (T3) ELISA Kit

Catalog No.: abx257133

Size: 96T

Range: 1.56 ng/ml - 10 ng/ml

Sensitivity: < 0.938 ng/ml

Storage: Store at 4°C for up to 6 months.

Application: For quantitative detection of T3 in Serum, Plasma, Tissue Homogenates and other biological fluids.

Introduction: Triiodothyronine, also known as T3, is a thyroid hormone. It affects almost every physiological process in the body, including growth and development, metabolism, body temperature, and heart rate. Production of T3 and its prohormone thyroxine (T4) is activated by thyroid-stimulating hormone (TSH), which is released from the anterior pituitary gland. This pathway is part of a closed-loop feedback process: Elevated concentrations of T3, and T4 in the blood plasma inhibit the production of TSH in the anterior pituitary gland. As concentrations of these hormones decrease, the anterior pituitary gland increases production of TSH, and by these processes, a feedback control system stabilizes the amount of thyroid hormones that are in the bloodstream. T3 is the true hormone. Its effects on target tissues are roughly four times more potent than those of T4. Of the thyroid hormone that is produced, just about 20% is T3, whereas 80% is produced as T4. Roughly 85% of the circulating T3 is later formed in the liver and anterior pituitary by removal of the iodine atom from the carbon atom number five of the outer ring of T4. In any case, the concentration of T3 in the human blood plasma is about one-fortieth that of T4. The half-life of T3 is about 2.5 days. The half-life of T4 is about 6.5 days.

Principle of the Assay

This kit is based on a competitive enzyme-linked immuno-sorbent assay technology. T3 is pre-coated onto 96-well plates. The standards, samples and a biotin conjugated antibody specific to T3 are added to the wells and incubated. After washing away the unbound conjugates, HRP Streptavidin (SABC) is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain T3 will produce a blue colour product that changes into yellow after adding stop solution. The intensity of the color yellow is inverse proportional to the T3 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of T3 can be calculated.

Kit components

1. One pre-coated 96 well plate
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 20 ml
4. Biotin conjugated antibody (Concentrated): 60 µl, Dilution 1:100
5. Antibody diluent buffer: 10 ml
6. HRP streptavidin conjugate (SABC) (Concentrated): 120 µl, Dilution 1:100
7. SABC diluent buffer: 10 ml
8. TMB substrate: 10 ml
9. Stop solution: 10 ml
10. Wash buffer (25X): 30 ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the blood at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

2. Wash buffer

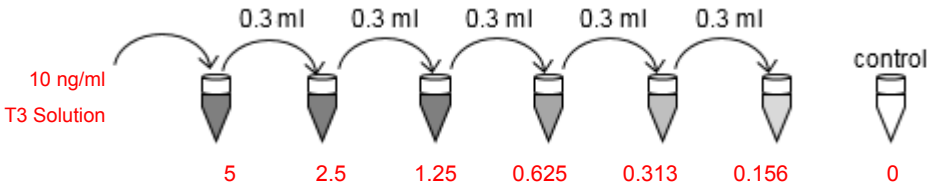
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

Preparation of the T3 standard: standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. **(Note: Do not dilute the standard directly in the plate).**

a.) 10 ng/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube, keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles.

b.) 5 ng/ml → 0.15625 ng/ml standard solutions: Label 6 tubes with 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml and 0.15625 ng/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 10 ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution can be stored at 4°C for up to 12 hours, or at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hour before the experiment.
a.) Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

5. Preparation of HRP Streptavidin Conjugate (SABC) working solution: prepare no more than 30 min before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the SABC with SABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of SABC into 99 µl of SABC diluent buffer.

B. Assay Procedure
Equilibrate the SABC working solution to room temperature and TMB substrate at 37°C, for at least 30 minutes prior to use. It is recommended to plot a standard curve for each test.

1. **Wash the plate two times before adding standard, samples and buffers.** Any strips that are not being used should be kept dry and stored at 4°C. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50 µl of the prepared standards solutions into the standard wells.
3. Add 50 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 50 µl of appropriately diluted sample into test sample wells.
5. Immediately add 50 µl of Biotin conjugated antibody working solution into each well. (Please add the solution at the bottom of each well without touching the side wall).
6. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 45 minutes.
7. Remove the cover, and wash the plate 3 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Fill each well completely with wash buffer and soak for at least 1-2 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of three times.

Please note: For automated washing, discard the solution in all wells and wash three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

8. Add 100 µl of HRP Streptavidin Conjugate (SABC) working solution into each well, cover the plate with a new sealer and incubate at 37°C for 30 minutes.
9. Remove the cover and wash the plate 5 times with Wash buffer. Allow the wash buffer to remain in the wells 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.
10. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the standard wells the reaction can be terminated.
11. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between T3 concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and absorbance measured (x-axis). Apply a best fit trendline through the standard points. Use this graph calculate sample concentrations based on their OD values. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1.Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.

2.It is recommended to assay all standards, controls and sample in duplicate.

3.Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.

4.Ensure plates are properly sealed or covered during incubation steps.

5.Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.

6.To avoid cross contamination do not reuse pipette tips and tubes.

7.Do not use components from a different kit or expired ones.

8.The substrate is supplied as a ready to use solution. The TMB substrate is light sensitive and should be protected from direct sunlight and UV sources. Unreacted substrate should be colorless or very light yellow in appearance. Equilibrate to room temperature (25°C) prior to use, aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of T3 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of T3 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

E. Typical Data & Standard Curve

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Concentration ng/ml	0	0.15625	0.3125	0.625	1.25	2.5	5	10
OD450	2.18	1.528	0.823	0.503	0.287	0.225	0.169	0.1