Total T3 (Triiodothyronine) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIATT31	Total T3 ELISA	96 Tests

Intended Use:

Total T3 ELISA is intended for quantitative determination of Total Triiodothyronine in human serum. This reagent is for In vitro diagnostic use by trained professionals only.

Summary and Principle:

Measurement of T3 is an important tool for the assessment of thyroid function. T3 determination is helpful in distinguishing between euthyroid and hyperthyroid subjects. T3 levels are elevated in women who are pregnant and in women receiving oral contraceptives or oestrogen treatment. T3 determination is an important factor in the diagnosis of thyroid diseases. Its measurement has uncovered a variant of hyperthyroidism in thyrotoxic patients with elevated T3 levels and normal T4 levels. An increase in T3 without an increase of T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients.
Total T3 ELISA assay is based on the principle of one step competitive method. The microwells are coated with anti-T3 antibody. Samples and enzyme labelled T3 antigen reagent are combined in the reaction. During incubation, the antigen in the conjugate competes with T3 present in the sample for limited binding sites on the solid phase anti-T3 antibody. After washing and addition of Substrate Solution, a chromogenic reaction takes place between the substrate and HRP label enzyme which is stopped and stabilised by the addition of Stop Solution. This results in a final yellow coloured solution, the colour intensity of which can be measured spectrophotometrically and is inversely proportional to the amount of TT3 in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with Anti-T3 antibody. The microwells can be broken and used separately. Place unused wells or strips in the plastic sealable bag provided together with the desiccant and store at 2 - 8°C. Once opened the wells are stable until expiry stored as described at 2 - 8°C.
T3 Calibrators	6x1ml	6 vials containing T3 in buffer matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS Once open stable until expiry at 2 - 8°C.
Conjugate Diluent	1x15ml	1 vial containing buffer matrix. Ready to use. Once open stable until expiry at 2 - 8°C.
Enzyme Conjugate (20X) Concentrate	1x0.8ml	1 vial containing T3 antigen conjugated to Horseradish Peroxidase in buffered saline. Once open, stable until expiry at 2 - 8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. concentrate must be diluted 1/50 with distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable until expiry at 2 - 8°C.
Stop Solution	1x12ml	Hydrochloric Acid solution (2N). Ready to use. Once open, stable until expiry at 2 - 8°C.

Traceability: The calibrators are manufactured using pure grade T3 and signal matched to our working calibrators.
Plastic sealable bag, IFU and plate covers.

Materials required but not provided:

Automatic microplate reader, microplate washer, distilled water, plate shaker, micropipettes, incubator, disposable reagent troughs.

Specimen Collection:

Collect serum by separation after standard venepuncture technique. TT3 is stable in serum for up to 7 days at 2 - 8 °C or for 1 month at -20 °C.
If sediments are present in samples they should be removed by centrifugation as their presence may interfere in the assay.
Do not use grossly haemolytic or lipemic samples.
Make sure samples, calibrators, and controls are brought to room temperature (18 - 25 °C) before measurement.

Storage and Stability:

Store unopened kits at 2 - 8°C at all times. Once kits are opened, place unused wells in the zip-lock plastic bag along with the desiccant and return to 2 - 8°C and tightly recap all reagent vials and return to 2 - 8°C. When stored under these conditions all kit components will be stable until the expiry date.

Precautions and Safety:

The ELISA assay is time and temperature sensitive. To avoid incorrect results, follow the test procedure steps exactly.
For in vitro diagnostic use only by trained professionals only.
All products that contain human serum or plasma have been tested and found to be non-reactive for HBsAg, HCV and HIV I/II. But all products should be regarded as potentially biohazardous in use and for disposal.
Mix the sample in the wells thoroughly by sharply tapping the side of the plate.
A thorough washing procedure is essential for obtaining accurate results. Use of an automatic plate washer is recommended. For each wash cycle leave the plate to soak for one minute. After the last wash cycle invert the plate onto absorbent paper and tap the plate to remove all remaining remnants of wash buffer.
Do not use reagents beyond the labelled expiry date.
Do not mix or use components from kits with different batch numbers.
The timing of substrate and stop solution additions across the plate should be the same for each reagent to eliminate differences in incubation time for the samples.

Procedure:

Reagent preparation:
Bring all samples, calibrators, and controls to room temperature (15 - 25 °C). Mix all reagents through gently inverting prior to use. Prepare Wash Solution by adding the contents of the Wash Buffer Concentrate bottle to 735 ml of distilled water.
Prepare a suitable volume of Conjugate Reagent for current assays, for example by diluting 200 µl Enzyme Conjugate Concentrate in 3.8 ml of Conjugate Diluent. The Conjugate Reagent will be stable up to 2 weeks stored at 2 - 8°C.
Calibrators may be supplied liquid, ready to use or lyophilised. If lyophilised, reconstitute each vial with 0.5 ml distilled water. Allow to stand for 20 minutes then mix well before use. After assay, recap tightly and store at 2 - 8°C.

STEP 1

Preparation: Remove the number of wells required and assign calibrators, controls and samples to well positions for the assay run.

STEP 2

Addition of samples and calibrators: Add 50 µl of calibrators, controls and samples to assigned wells. Shake the plate for 10 seconds to distribute samples.

STEP 3

Addition of Conjugate Reagent: Add 100 µl of Conjugate Reagent to each well. Shake the plate for 30 seconds to ensure that the added components are well mixed.

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at room temperature (19 - 22°C).

STEP 5

Wash Step: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted Wash Solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 6

Addition of the Substrate: Add 100 µl of Substrate Solution to each well. Gently shake the plate for 5 seconds to mix.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature (15 - 25°C). Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 100 µl of Stop Solution to each well and mix gently until the well contents change from blue to yellow.

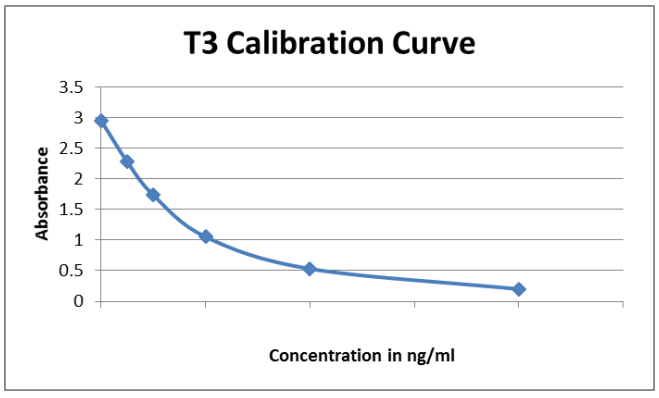
STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. The results should be read within 30 minutes of adding the Stop Solution. Record the absorbance values for each well.

Calculation of results:

Calculate the mean absorbance for any duplicate well measurements. A 4-Parameter curve fit is recommended to generate a calibration curve. If no statistical method is available, manually plot the absorbance against concentration for each calibrator. Draw the best-fit curve through the plotted points on linear graph paper.

Example:



Note:

The TT3 ELISA assay is particularly sensitive to temperature. If incubations are carried out at temperatures higher than 19 – 22°C, it may be necessary to increase the dilution of the Conjugate Reagent (1/40) or reduce incubation time.

Quality Control:

Each laboratory should run Quality Controls in each assay run covering the assay range. Results for unknown samples tested are valid if the Quality Control values fall within the assigned concentration ranges for each level.

Limits and ranges
Measuring range
0.25 - 10.0 ng/ml (defined by the lower detection limit and the maximum of the master curve).

Sensitivity
Lowest detection limit: 0.25 ng/ml.
The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard.

Expected values
0.60 - 2.0 ng/mL
Each laboratory should determine a reference range pertinent to its own population.

Performance Characteristics:

Precision
Precision was determined using three levels of pooled human sera for 20 replicates within one plate and for 20 replicates run on separate plates. The following results were obtained:

Intra assay Precision:

Pool	Mean	SD	CV%
1	0.79	0.068	10.88
2	2.54	0.11	4.26
3	6.45	0.27	4.10

Inter assay Precision:

Pool	Mean	SD	CV%
1	0.68	0.079	11.61
2	2.11	0.13	5.97
3	5.73	0.34	6.01

Interferences
Enzyme immunoassays potentially demonstrate interference by samples containing rheumatoid factor and antinuclear antibodies, and samples from patients receiving treatments containing mouse monoclonal antibodies. The reagents have been formulated to reduce such interference, but the effects may not be eliminated completely.

Limitations:
The result from this test should not be used as the sole criteria for diagnosis, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

References:

1. Walker WHC. Clin Chem 1977; 23: 384.
2. Kirkegaard C, Friis T and Siersback-Nielsen K. Acta Endocrinol 1974; 77: 71.
3. Wisdom GB. Clin Chem 1976; 22: 1243.
4. Hoffenberg R. Medicine 1078; 8: 392.
5 Lieblich J and Utiger RD. J Clin Invest 1972; 51: 1939.

REF	Catalogue number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by Date
	Manufacturer		

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