Free T4 (Free Thyroxine) ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAFT41	Free T4 ELISA	96 Tests

Intended Use:
The Free T4 ELISA (FT4) is intended for quantitative measurement of Free Thyroxine in human serum. This reagent is for *in vitro* diagnostic use only.

Summary and Principle:
Free Thyroxine is an indicator of thyroxine activity in the body. Under normal thyroid conditions, as the concentrations of the carrier proteins alter, total T4 levels change so that the FT4 concentration remains constant. Measurement of FT4, thus, correlates better with clinical status than total T4 status.

The Free T4 ELISA assay is based on the principle of competition. Microwells are coated with T4 antigen. Free T4 antigen in the sample competes with the microwell coated T4 antigens for binding sites on the HRP-labelled anti-T4 antibody in the conjugate. A washing step removes unbound sample material and conjugate. Addition of Substrate consisting of TMB and peroxide results in a chromogenic reaction catalysed by the HRP on bound antibody giving rise to development of a blue colour. The reaction is stopped with the addition of acidic reagent and the colour is changed to yellow. The colour intensity is inversely proportional to the concentration of FT4 in the sample and can be measured spectrophotometrically.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with T4 antigen. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2 - 8°C. Once open the wells are stable for 2 months at 2 - 8°C.
FT4 Calibrators	6x1ml	6 vials containing T4 (pmol/l) made up in a human serum matrix. The exact concentrations are provided on the vial labels and not listed here since concentrations are subject to change. Ready to use. Once open stable for 1 month at 2 - 8°C.
Enzyme Conjugate	1x6ml	1 vial containing HRP labelled mouse monoclonal anti T4 antibody in buffered saline. Once open, stable for 2 months at 2 - 8°C.
Wash Buffer Concentrate (40X)	1x25ml	PBS-Tween at pH 7.4, 40X concentrate. Concentrate must be diluted 1 to 40 with distilled water before use. Once diluted it is stable at room temperature for 2 months.
Substrate Solution	1x11ml	TMB and hydrogen peroxide reagent. Ready to use. Once open, stable for 2 months at 2 - 8°C.
Stop Solution	1x6ml	Sulphuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2 - 8°C.

Traceability: The calibrators are matched to a working calibrator. The working calibrator is manufactured by gravimetric methods by the addition of T4 antigen to hormone free human serum.
Also Provided: IFU, resealable bag, plate covers.

Materials required but not provided:
Distilled water, micropipettes, incubator, 96-well plate reader and 96-well plate washer, absorbent paper.

Sample Collection:
Collect serum samples by separation from red blood cells after standard venepuncture technique. Store samples at 15 - 25°C for up to 8 hours, for 3 days at 2 - 8oC and 1 month at -20oC, under which conditions total T4 will be stable with a recovery within 90-110%. Avoid more than one freeze-thaw. Some sample collection tubes may contain differing substances which could affect the test result. If samples contain precipitate centrifuge before use. Do not use heat-inactivated samples. Do not use samples and controls stabilised with azide. Avoid grossly haemolytic, lipemic or turbid samples. Ensure that complete clot formation in serum samples has taken place prior to centrifugation otherwise the presence of fibrin may cause erroneous results. If in doubt, centrifuge the samples.

Storage and Stability:
The contents of the kit will remain stable up to expiry date when stored unopened at 2 - 8oC. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag provided and return to 2 - 8oC, under which conditions the wells will remain stable for 2 months, or until the expiry date, whichever is earlier. Seal and return all the other unused reagents to 2 - 8oC, under which conditions the stability will be retained for 2 months, or until the expiry date, whichever is earlier.

Precautions:
The ELISA assay is time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify the steps. Reliability of results cannot be guaranteed if there are deviations from the instructions.
The calibrators contain human serum based components. They have been tested and found to be non-reactive to HBsAg, HIV and HCV antibodies and syphilis. The assay contains materials of animal origin including BSA which have been sourced from countries where BSE has not been reported. However, it is recommended that all the reagents be handled as if potentially infectious and care to be taken in their use and disposal.
Wear laboratory protective equipment including gloves and safety glasses whilst handling reagents, controls and samples. Wash hands thoroughly after each operation.
Samples and reagent additions to wells should not introduce any bubbles as these may cause erroneous results.
Use new pipette tips for each sample and reagent addition to avoid cross contamination.
Do not use kits beyond the expiry date.
Do not interchange components from other kits.
The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore the substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

Procedure:
Reagent preparation:
Ensure the patients' samples, calibrators, and controls are at room temperature (15 - 25 °C) before beginning the assay. Mix all reagents gently before use. Prepare wash solution concentrate by adding the contents of the bottle to 975 ml distilled water or dilute a portion by 1/40. Stable for 2 months at room temperature. Do not use Substrate if it looks blue. Do not use reagents that are contaminated.

STEP 1
Preparation: Remove the number of wells required and assign each well for the calibrators, controls and samples

STEP 2
Addition of Samples: Add 50 µl of calibrators, controls and samples to each well.

STEP 3
Addition of Enzyme Conjugate: Add 50 µl of Enzyme Conjugate solution to each well. Mix well by tapping the edge of the plate gently.

STEP 4
Incubation: Cover the plate with a plate cover and incubate for 60 minutes at 37°C.

STEP 5
Washing: 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. Use of an automated microplate strip washer is recommended. 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.

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 and do not shake the plate after the substrate addition.

STEP 8
Stopping the Reaction: Add 50 µl of Stop solution to each well and mix gently. Shake the plate until the well contents change completely 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.

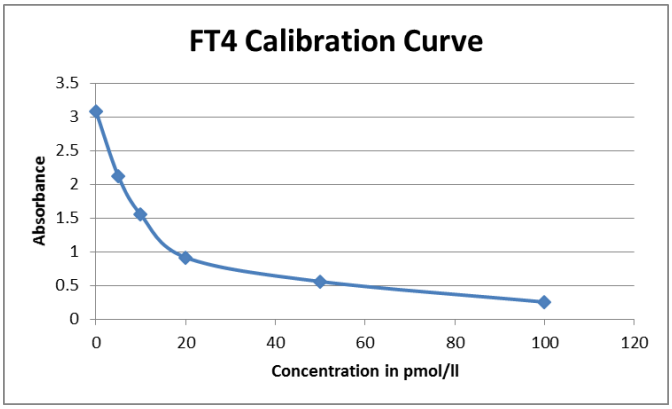
Calculation of results:
Record the absorbances obtained from the microplate reader. Ensure that mean Absorbance values are calculated for duplicate measurements.
Plot the Absorbance on the y axis and Concentration in pmol/l on the x axis.
Draw a point to point curve through the plotted points on a linear graph paper.
To determine the concentration of an unknown sample, locate the absorbance of the sample on the y axis and find the intersecting point on the curve. Read the concentration from the x axis by dropping a line from the intersecting point of the absorbance on the curve.

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	3.084	0 pmol/l
CAL B	2.121	5 pmol/l
CAL C	1.842	10 pmol/l
CAL D	0.915	20 pmol/l
CAL E	0.557	50 pmol/l
CAL F	0.253	100 pmol/l
Control Level 1	1.824	10.29 pmol/l
Control Level 2	0.795	33.38 pmol/l
Sample	1.408	17.02 pmol/l

Calculation
Where calibrator details are entered into the microplate reader, the analyser automatically calculates the analyte concentration of each sample.
Conversion factors:
pmol/l x 0.0777 = ng/dl
ng/dl x 12.872 = pmol/l
pmol/l x 0.777 = ng/l



Quality Control:
Each laboratory should run quality controls at levels in the low, normal, and elevated range for monitoring assay performance. The concentration results of each quality control should fall within the assigned range for the analyte.

Limitations:
Performance of this test has not been established with neonatal samples.
In NTI (severe nonthyroidal illness), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction.
Serum FT4 values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
The interpretation of FT4 is complicated by a variety of drugs that can affect the binding of T4 to the thyroid hormone carrier proteins or interfere with its metabolism to T3.
In rare conditions associated with extreme variations in albumin binding capacity for T4-such as familial dysalbuminemic hyperthyroxinemia direct assessment of FT4 may be misleading.
Circulating antibodies to T4 and hormone binding inhibitors may interfere with the performance of the assay.
If patient results read higher than the measuring range, only report as > 100 pmol/l and do not try to dilute the samples for remeasurement. TBG variations in different matrices will not allow FT4 hormone to dilute serially.
A decrease in FT4 values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates.
Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human antimouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
The Free T4 result from this test should not be used as the sole criteria for the diagnosis of thyroid status, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

Performance Characteristics:

Interference:
The assay is unaffected by icterus (bilirubin < 600 µmol/l or < 35 mg/dl), haemolysis (Haemoglobin < 0.559 mmol/l or < 0.9 g/dl), lipemia (Intralipid < 1200 mg/dl), and biotin < 94 nmol/l or < 23 ng/ml based on Recovery within ± 10 % of initial value.
Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. . Samples from patients on therapies involving animal derived products are not suitable to be tested by this assay.

Measuring range
2 - 100 pmol/l or 0.155 - 7.77 ng/dl (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 2 pmol/l or 0.155 ng/dl. Values above the measuring range are reported as > 100 pmol/l or 7.77 ng/dl.

Sensitivity
Lower detection limit: 2 pmol/l or 0.155 ng/dl
The detection limit represents the lowest analyte level that can be distinguished from zero.

Expected values
11.5 - 23.8 pmol/l or 0.893 - 1.849 ng/dl
These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 777 healthy test subjects using the Free T4 ELISA. The test panel did not include samples from children, adolescents or pregnant women so the reference range may not be applicable to these groups. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference ranges.

Precision
Precision was determined using reagents, pooled human sera, and controls testing 2 times daily for 20 days (n= 40). The following results were obtained for Intra Assay precision:

Panel	Mean	SD	CV%
Serum pool 1	14.36	1.10	7.66
Serum pool 2	28.51	1.95	6.84
Serum pool 3	45.44	2.96	6.51
Control Level 1	17.33	1.28	7.39
Control Level 2	32.15	2.31	7.19

Method comparison
A comparison of the FT4 assay (y) with another commercial Free T4 ELISA (x) using clinical samples (sample concentration range was approximately 4 to 100 pmol/l) gave the following Linear Regression (n = 91):

y = 1.060 x - 1.524, r = 0.970

Specificity
For the antibody derivative used, the following cross-reactivities were found: L-T4 and D-T4 100 %; L-T3 1.89 %; D-T3 1.44 %; 3-iodo-L-tyrosine 0.002 %; 3,5-diiodo-L-tyrosine 0.008 %.

References:
1. Wheeler MH, Lazarus JH. Diseases of the Thyroid. London, Glasgow, Weinheim, New York, Tokyo, Melbourne, Madras: Chapman and Hall, 1994;107-115.
2. Pfannenstiel P, Saller B. Schilddrüsenkrankheiten Diagnose und Therapie. Berliner Medizinische Verlagsanstalt GmbH 1991;2:43-62,72-89.
3. Ekins RP. Measurement of free hormones in blood. Endocr Rev 1990;11:5.
4. Ekins RP, Ellis SM. The radioimmunoassay of free thyroid hormones in serum. In Robbins J, Braverman LE (eds). Thyroid research, Proceedings of the Seventh International Thyroid Conference, Boston. Amsterdam, Excerpta Medica 1975:597.

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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