Free T4 (Free Thyroxine) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAFT41	Free T4 Elisa	96 Tests

Intended Use:

Free T4 Elisa 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 a indicator of thyroxine activity in the body. Under normal thyroid conditions, as the concentrations of the carrier proteins alter, the 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 FT4 assay is based on a one step competitive method. The sample, T4 coated microwells and enzyme labelled Anti T4 are combined in the reaction. During the incubation, T4 coated on microwells, the FT4 present in the sample, compete for binding sites in the enzyme labelled antibodies. After washing and addition of substrate solution, there results a chromogenic reaction which is stopped by the addition of a stopping solution. This results in a final yellow coloured solution which is measured in a Elisa Reader. The colour intensity is inversely proportional to the amount of FT4 in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with T4 analogue. 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 at concentrations of 0.0, 5, 10, 25, 50 and 100 pmol/l (1pmol/l x 0.0775 = 1 ng/dl) made up in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once open stable for 1 month at 2-8°C.
Enzymatic Conjugate	1x11ml	1 vial containing 11ml of HRP labelled mouse monoclonal Anti T4 antibodies 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. Once open, stable for one month at 2-8°C. The concentrate must be diluted 1 to 40 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 for one month at 2-8°C.
Stop Solution	1x7.5ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 1 month 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: Plastic Sealable bag, IFU and Cardboard plate covers.

Materials required but not provided:

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

Specimen Collection:

Serum is the sample of choice. Collect serum samples in accordance with correct medical practices. Ensure that the samples are clear and do not have suspended particles or sediments. Avoid usage of highly lipaemic, haemolytic or turbid samples. Samples are stored at room temperature up to 8 hours and store those not required for assay within 8 hours at 2-8°C. Those samples that are not performed within 8 hours, have to be stored at -20°C. Avoid multiple freeze thaw cycles. After thawing, bring to room temperature and mix well by gentle shaking.

Storage and Stability:

1. Store all components at 2-8°C. Do not freeze. Avoid strong light.
2. Place unused wells in the zip-lock bag with desiccant provided, the seal the zip-lock bag in the aluminium foiled pouch with a plate lid and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the labelled expiry date, whichever is earlier.
3. Seal and return unused calibrators to 2-8°C, under which conditions the stability will be retained for 1 months, for longer use, store opened calibrators in aliquots and freeze at -20°C. Avoid multiple freeze thaw cycles.
4. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained for 2 months, or until the expiry date whichever is earlier. whichever is earlier.

Precautions and Safety:

The Elisa assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify them.

1. **For professional use only.**
2. **Follow the instructions in this IFU as reliability of results cannot be guaranteed if there are deviations from the instructions.**
3. **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 like BSA which have been sourced from countries where BSE has not been reported. It is recommended that all human serum based material may be considered potentially infectious and care to be taken in their use.**
4. **Wear laboratory protection equipment such as gloves, glasses whilst handling reagents, controls and samples. Wash hands thoroughly after each operation.**
5. **Samples in the microwells should not have bubbles as these bubbles may result in erroneous results.**
6. **Wash the wells completely. Avoid overflow during wash. Remove any residual wash buffer by tapping the microwells on a clean towel or absorbent paper. It is ideal to use an automated microplate washer.**
7. **Use new pipette tips for each pipetting to avoid cross contamination.**
8. **Do not use kits after expiry date**
9. **Do not interchange components from other kits.**
10. **Once diluted the Enzyme conjugate solution is stable for 7 days at 4°C. Dilute enough enzyme conjugate concentrate as required for the assay series.**
11. **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.**
12. **If more than one plate is used, it is recommended that the calibration curve is repeated.**
13. **Secure the calibrator vial caps, if unused calibrators are stored for further use.**
14. **It is important that the time of reaction in each swell is held constant to achieve reproducible results.**
15. **It is important to calibrate all equipment e.g. micropipettes, microplate readers, automated microplate strip washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.**
16. **Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate.**
17. **Failure to remove adhering solution adequately in the washing step will lead to erroneous results.**
18. **Use no more than 32 wells for each assay run, when manual pipette is used. Complete pipetting of all calibrators, controls and samples within 5 minutes.**

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature prior to use.
2. Adjust the incubator to 37°C.
3. Add 1 volume of the wash fluid concentrate to 39 volumes of distilled water to give the required volume and mix well. Once diluted the wash solution is stable for 2 months at room temperature.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series.

STEP 2

Addition of Samples and calibrators: Add 50ul of Calibrators and Samples to each well.

STEP 3

Addition of Enzyme Conjugate: Add 100ul of the diluted Enzyme Conjugate solution 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 37°C.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer of 350ul. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the Substrate: Add 100ul of Substrate Solution to each well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 8

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently. Shake the plate to mix till the solution changes to yellow from blue.

STEP 9

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader. Note down the absorbances.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance in Y axis and Concentration in pmol/l in 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.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	2.984	0 pmol/l
CAL B	2.006	5 pmol/l
CAL C	1.234	10 pmol/l
CAL D	0.455	25 pmol/l
CAL E	0.208	50 pmol/l

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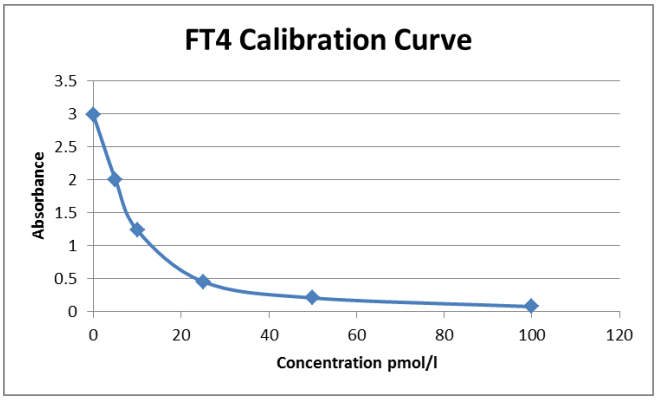
CAL F	0.075	100 pmol/l
Control Level 1	1.164	11.35
Control Level 2	0.430	27.53
Sample	0.975	15



Quality Control:

It is recommended that each test run should be accompanied with quality controls. Recommended controls are QCCIAL1, QCCIAL2, QCCIAL3 – Immunoassay Controls L1, L2 and L3.

Performance Characteristics:

1. Intra assay Precision:

Two human serum panels were run in replicates of 20. Following are the results:

Panel	Data no.	Mean	SD	CV%
1	20	18.42	0..38	2.08
2	20	32.5	0.95	2.91

2. Inter assay Precision:

Two human serum panels in replicates of 2 across 20 days. Following are the results:

Panel	Data no.	Mean	SD	CV%
1	40	14.97	1.04	6.94
2	40	22.11	0.62	2.85

3. Sensitivity:

Analytical sensitivity was found to be </= 2.5 pmol/l. Analytical sensitivity is the concentration corresponding to the mean absorbance of 10 replicates of the Calibrator A minus 2 standard deviations.

4. Analytical Specificity:

The cross reactivity of this assay against T4 and rT3 was determined by spiking concentrations of the potential interferents to Calibrator A. The results are provided below:

Interferent	Concentration	Measure value in pmol/l
T3	500 ng/ml	4.49
rT3	200 ng/ml	5.29

5. Correlation:

Correlation studies were undertaken using a commercially available predicate assay. The data is summarized below:

Method	Data points	Intercept	Slope	Correlation coefficient
Linear Regression	219	0.4192	0.9877	0.985

Reference range:

It is recommended that each laboratory establish its own normal reference ranges for the population that it serves.

Reference range for Adults: 8.5 pmol/l to 22.5 pmol/l.

Limitations:

- The assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
- If the results are inconsistent with clinical findings, additional testing is suggested o confirm the results.
- Heterophilic antibodies and RF 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.
- This assay is not suitable for neonatal samples.
- Serum FT4 concentrations depend upon a variety of factors: function of hypothalamus and its regulation, TBG concentration and binding of FT4 to TBG. A single result of FT4 is not sufficient to assess the clinical status of the patient.
- In Non-thyroidal illnesses, the assessment of thyroid status is carried out using a variety of assays. TSH is also recommended to be performed.
- Serum FT4 concentration may be elevated under conditions such as pregnancy or administration of Oral contraceptives.
- Direct assessment of FT4 may be misleading in extreme variations in albumin binding capacity for T4 in conditions such as Familial dysalbuminemic hyperthyroxinemia).
- Circulating antibodies to T4 and hormone binding inhibitors may interfere with the performance of the assay.
- Samples with values higher than the highest calibrator must be reported as >100 pmol/l. Do not dilute the samples. TBG variations in different matrices will not allow FT4 to dilute serially.
- Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop Human anti mouse antibodies. HAMA can produce falsely high or falsely low values in Immunoassays using mouse monoclonal antibodies. This assay is not suitable for samples containing HAMA.
- A decrease in FT4 is found in protein wasting disease such as liver disease, administration of testosterone, diphenylhydantoin or salicylates. Refer Young D.S, Effects of drugs on clinical laboratory tests for more information.

References:

1. Dayan CM. Interpretation of thyroid function tests. *Lancet*. 2001;357(9256):619-624.

2. Nelson JC. Underestimates of serum free thyroxine (T4) concentrations by free T4 immunoassays. *Journal of Clinical Endocrinology & Metabolism*. 1994;79(1):76-79.

3. Lee RH, Spencer CA, Mestman JH, et al. Free T4 immunoassays are flawed during pregnancy. *American Journal of Obstetrics and Gynecology*. 2009;200(3):260.e1-260.e6.

4. Kinders RJ, Hass GM. Interference in immunoassays by human anti-mouse antibodies. *Eur. J. Cancer*. 1990;26(5):647-648.

5. Spencer C, Takeuchi M, Kazarosyan M, et al. Interlaboratory/intermethod differences in functional sensitivity of immunometric assays of thyrotropin (TSH) and impact on reliability of measurement of subnormal concentrations of TSH. *Clin Chem*. 1995;41(3):367-374.

6. Young DS. Effects of drugs on clinical laboratory tests. *Ann. Clin. Biochem*. 1997;34 (Pt 6):579-581.

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

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