GBLISA FT3 Kit

KIT NAME	KIT SIZE	CAT. NO
GBLISA FT3 Kit	96T	GBLFT3096T

GENUINE BIOSYSTEM

INTENDED USE

Immunoassay for the in vitro quantitative determination of free triiodothyronine in human serum.

SUMMARY

Triiodothyronine is one of the thyroid hormones present in serum which regulates metabolism. Determination of this hormone concentration is important for the diagnostic differentiation of euthyroid, hyperthyroid, and hypothyroid states. The major fraction of total triiodothyronine is bound to the transport proteins (TBG, prealbumin, albumin). Free triiodothyronine (fT3) is the physiologically active form of the thyroid hormone triiodothyronine (T3). The determination of free T3 has the advantage of being independent of changes in the concentrations and binding properties of the binding proteins; additional determination of a binding parameter (T-uptake, TBG) is therefore unnecessary. (1, 2, 3) In normal thyroid function, as the concentrations of the carrier proteins alter, the total T3 level changes so that the FT3 concentration remains constant. (4) Thus, measurements of FT3 concentrations correlate more reliably with clinical status than total T3 levels. For example, the increase in total T3 levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T3 levels while the FT3 con- centration remains basically unchanged. (5) In addition, it has been found that the mean FT3 value has a gradient decreasing from young to older. (6)

TEST PRINCIPLE

Competition principle. Total duration of assay: 80 minutes.• Sample, T3 derivant coated microwells and enzyme labeled Anti-T3 are combined. • During the incubation, T3 derivant coated on microwells and FT3 present in the sample compete for binding to the enzyme labeled antibodies. • After washing, a complex is generated between the solid phase and enzyme-linked antibodies by immunological reactions. • Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance. • The color intensity is inversely proportional to the amount of FT3 in the sample.

REAGENTS

Materials provided

• Coated Microplate, 8 x 12 strips, 96 wells, pre-coated with T3 derivant
• Calibrators, 6 vials, 1 ml each, ready to use; Concentrations: 0(A), 2(B), 5(C), 10(D), 20(E) and 50(F) pmol/L.
• Enzyme Conjugate, 1 vial, 6.0 ml of HRP (horseradish peroxidase) labeled sheep monoclonal Anti-T3 in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.2% ProClin300 preservative.
• Substrate, 1 vial, 11ml, ready to use, (tetramethylbenzidine) TMB.• Stop Solution, 1 vial, 6.0 ml of 1 mol/l sulfuric acid.
• Wash Solution Concentrate, 1 vial, 25 ml (40X concentrated), PBS-Tween wash solution.
• IFU, 1 copy
• Plate Lid 1 piece.

Materials required (but not provided)

• Microplate reader with 450nm and 620nm wavelength absorbent capability.
• Microplate washer.
• Incubator
• Plate shaker.
• Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
• Absorbent paper
•Distilled water

PRECAUTIONS AND WARNINGS

• For in vitro diagnostic use only. For professional use only.
• All products that contain human serum or plasma have been found to be non-reactive for HBsAg, HCV and HIVI/II. But all products should be reared as potential biohazards in use and for disposal.
• Mix the sample in the wells thoroughly by shaking and eliminate the bubbles.
• Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
• Wash the wells completely. Each well must be fully injected with wash solution. The strength of injection, however, is not supposed to be too intense to avoid overflow. In each wash cycle, dry the liquids in each well. Strike the microplate onto absorbent paper to remove residual water droplets. It is recommended to wash the microplate with an automated microplate strip washer.
• Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
• Do not use reagents beyond the labeled expiry date.
• Do not mix or use components from kits with different batch codes.
• If more than one plate is used, it is recommended to repeat the calibration curve.
• It is important that the time of reaction in each well is held constant to achieve reproducible results.
• Ensure that the bottom of the plate is clean and dry.
• Ensure that no bubbles are present on the surface of the liquid before reading the plate.
• The substrate and stop solution should be added in the same sequence to eliminate any time deviation during reaction.

STORAGE

• Store at 2-8℃.
• Place unused wells in the zip-lock aluminum foiled pouch and return to 2-8 °C, under which conditions the wells will remain stable for 2 months, or until the labeled expiry date, whichever is earlier.
• Seal and return unused calibrators to 2-8 °C, under which conditions the stability will be retained for 1 month, for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles
• Seal and return all the other unused reagents to 2-8 °C, under which conditions the stability will be retained for 12 months, or until the labelled expiry date, whichever is earliest.

SPECIMEN COLLECTION AND PREPARATION

• Collect serum samples in accordance with correct medical practices.
• Cap and store the samples at 18-25 °C for no more than 8 hours. Stable for 3 days at 2-8 °C, and 1 month at -20 °C. Recovery within 90-110 % of serum value or slope 0.9-1.1. Freeze only once.
• The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
• Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement.
• Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
• Avoid grossly hemolytic, lipemic or turbid samples.
• Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
• If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
• Ensure the patients' samples, calibrators, and controls are at ambient temperature (18-25 °C) before measurement. Mix all reagents through gently inverting prior to use.
• Adjust the incubator to 37 °C.
• Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
• Don's use Substrate if it looks blue.
• Don't use reagents that are contaminated or have bacteria growth.

QUALITY CONTROL

Eac**h laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as u**nknowns and values determined in every test procedure performed. The recommended controls requirement for this assay are to purchase trueness control materials separately and test them together with the samples within the same run. The result is valid if the control values fall within the concentration ranges printed on the labels.

TEST

Use only the number of wells required and format the microplates' wells for each calibrator and sample to be assayed.

- Add **50 µL** of **calibrators** or **samples** to each well.
- Add **50 µL** of **enzyme conjugate** to each well.
- Shake the microplate gently for **30 seconds** to mix.
- Cover the plate with a plate lid and incubate at **37 °C** for **60 minutes**.
- Discard the contents of the micro plate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
- Add **350 µl** of **wash solution**, decant (tap and blot) or aspirate. Repeat 4 additional 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.
- Add **100 µl** of **substrate** to each well.
- Incubate at **ambient temperature (18-25℃)** in the dark for reaction for **20 minutes**. Do not shake the plate after substate addition.
- Add **50 µl** of **stop solution** to each well.
- Shake for **15-20 seconds** to mix the liquid within the wells. It is important to ensure that the blue color changes to yellow completely.
- Read the absorbance of each well at **450 nm** (using 620 to 630 nm as the reference wavelength to minimize well imperfections) in a micro plate reader. The results should be read within **30 minutes** of adding the stop solution.

CALCULATION

- Record the absorbance obtained from the printout of the microplate reader.
- Calculate the mean absorbance of any duplicate measurements and use the mean for the following calculation.
- Plot the common logarithm of absorbance against concentration in pmol/L for each calibrator.
- Draw the best-fit curve through the plotted points on linear graph paper. Point-to- Point method is suggested to generate a calibration curve.The following data is for demonstration only and cannot be used in place of data generations at the time of assay

SAMPLE	VALUE (pmol/L)	Absorbance
Calibration A	0	3.057
Calibration B	2	1.871
Calibration C	5	1.431
Calibration D	10	1.018
Calibration E	20	0.642
Calibration F	50	0.240
Control 1	4.83	1.456
Control 2	15.81	0.876
Sample	5.42	1.396

LIMITATIONS – INTERFERENCE

- The assay is unaffected by icterus (bilirubin < 600 µmol/L or < 35 mg/dL), hemolysis (Hb < 0.559 mmol/L or < 0.9 g/dL), lipemia (Intralipid < 1200 mg/dL), and biotin < 94 nmol/L or < 23 ng/mL
- Criterion: 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. This kind of samples is not suitable to be tested by this assay.
- Performance of this test has not been established with neonatal samples.
- In severe NTI (nonthyroidal illness), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction.
- Familial dysalbuminemic conditions may yield erroneous results on direct free T3 assays.
- If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 50 pmol/l). Do not try to dilute the sample. TBG variations in different matrices will not allow FT3 hormones to dilute serially.

CALCULATION

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L, pg/mL or ng/dL).

Conversion factors:
pmol/L x 0.651 = pg/mL
pg/mL x 1.536 = pmol/L
pg/mL x 0.1 = ng/dL

LIMITS AND RANGES

Measuring Range

0.5-50.0 pmol/L or 0.325-32.55 pg/mL(defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 0.5 pmol/L or < 0.325 pg/mL. Values above the measuring range are reported as > 50.0 pmol/L or 32.55 pg/mL.

Lower limits of measurement

Lower detection limit

Lower detection limit: 0.5 pmol/L or 0.325 pg/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 (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Expected values

3.3-7.5 pmol/L

These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 851 healthy test subjects examined.We have not studied the reference intervals in children, adolescents and pregnant women.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using GB reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 2 times daily for 20 days (n = 40). The following results were obtained:*

		Repeatability		Intermediate Precision	
Sample	Mean	SD pmol/L	CV %	SD pmol/L	CV %
Serum 1	5.77	0.39	6.64	0.50	8.72
Serum 2	13.88	0.79	5.71	1.03	7.46
Serum 3	28.6	1.56	5.46	2.09	7.31
PC universal 1	4.48	0.31	7.02	0.31	6.88
PC universal 2	12.15	0.81	6.66	0.84	6.93

Repeatability = within-run precision

METHOD COMPARISON

A comparison of the FT3 assay (y) with the Roche Cobas FT3 (x) using clinical samples gave the following correlations: Number of samples measured: 121

Linear regression y = 1.0407x - 0.2867 r = 0.9768

The sample concentrations were between approx. 2.5 and 40 pmol/L.

ANALYTICAL SPECIFICITY

For the antibody derivative used, the following cross-reactivities were found: D-T3 100 %; L-T4 < 0.31 %; D-T4 < 0.45 %; L-rT3 < 0.05 %; L-T2 < 0.8 %.

References1.

References

1. Wheelers MH, Lazarus JH. Diseases of the Thyroid. London, Glasgow, Weinheim, New York, Tokyo, Melbourne, Madras: Chapman and Hall Medical,1994:107-115.
2. Pfannenstiel P, Saller B. Schilddrüsenkrankheiten Diagnose und Therapie. Berliner Medizinische Verlagsanstalt GmbH 1995;2:30-32,60-62.
3. Fisher DA. Physiological variations in thyroid hormones; physiological and pathophysiological considerations. Clin Chem 1996;42:135-139.
4. Bartalena L, Robbins J. Variations in Thyroid Hormone Transport Pro- teins and Their Clinical Implications. Thyroid. 1992;2(3):237-245.
5. Carrero JJ, Qureshi AR, Axelsson J, et al. Clinical and biochemical im-plications of low thyroid hormone levels (total and free forms) in euthy- roid patients with chronic kidney disease. J. Intern. Med. 2007;262(6):690-701. 6.

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