GBLISA FT4 Kit

KIT NAME	KIT SIZE	CAT. NO
GBLISA FT4 Kit	96T	GBLFT4096T

GENUINE BIOSYSTEM

INTENDED USE

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

SUMMARY

The thyroid hormone thyroxine (T4) is physiologically part of the regulating circuit of the thyroid gland and has an effect on general metabolism. The major fraction of the total thyroxine is bound to transport proteins (TBG, prealbumin, and albumin). The free thyroxine (fT4) is the physiologically active thyroxine component.The determination of free thyroxine is an important element in clinical routine diagnostics. Free T4 is measured together with TSH when thyroid function disorders are suspected. The determination of fT4 is also suitable for monitoring thyrosuppressive therapy.The determination of free T4 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.

TEST PRINCIPLE

Competition principle. Total duration of assay: 80 minutes.• Sample, T4 derivant coated microwells and enzyme labeled Anti-T4 are combined. • During the incubation, T4 derivant coated on microwells and FT4 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 FT4 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), 5(B), 10(C), 20(D), 50(E) and 100(F) pmol/L.
• Enzyme Conjugate, 1 vial, 6.0 ml of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-T4 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.
• Plate Lid: 1 piece.Materials

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

Each laboratory should have assay controls at levels in the low, normal, and elevated range for monitoring assay performance. The controls should be treated as unknowns 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 (18-25ºC)** 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 assay202012

SAMPLE	VALUE (pmol/L)	Absorbance
Calibration A	0	3.084
Calibration B	5	2.121
Calibration C	10	1.842
Calibration D	20	0.915
Calibration E	50	0.557
Calibration F	100	0.253
Control 1	10.29	1.824
Control 2	33.38	0.795
Sample	17.02	1.408

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 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 FDH (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 a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 100 pmol/l). Do not try to dilute the samples. 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. A table of interfering drugs and conditions, which affect FT4 values, has been compiled by the Journal of the American Association of Clinical Chemists.
• Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human Anti-mouse 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.

CALCULATION

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

Conversion factors:
pmol/L x 0.077688 = ng/dL
ng/dL x 12.872 = pmol/L
pmol/L x 0.77688 = ng/L

LIMITS AND RANGES

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.

Lower limits of measurement

Lower detection limit

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. 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

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 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	14.36	1.10	7.66	1.25	8.72
Serum 2	28.51	1.95	6.84	2.13	7.46
Serum 3	45.44	2.96	6.51	3.32	7.31
PC universal 1	17.33	1.28	7.39	1.19	6.88
PC universal 2	32.15	2.31	7.19	2.23	6.93

Repeatability = within-run precision

METHOD COMPARISON

A comparison of the FT4 assay (y) with the Roche Cobas FT4 (x) using clinical samples gave the following correlations: Number of samples measured: 91

Linear regression y = 1.0595x – 1.5237, r = 0.9695

The sample concentrations were between approx. 4 and 100 pmol/L.

ANALYTICAL 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 %.

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