GBLISA TSH Kit

KIT NAME	KIT SIZE	CAT. NO
GBLISA TSH Kit	96T	GBLTSH096T

INTENDED USE
Immunoassay for the in vitro quantitative determination of thyrotropin in human serum.

SUMMARY
Thyroid-stimulating hormone (TSH, thyrotropin) is a glycoprotein having a molecular weight of approx. 30000 daltons and consisting of two subunits. Measurement of the serum concentration oftentimes thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism (1,2).TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism .Further, the advent of immunoenzymometric assays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provide the means for discrimination in the hyperthyroid-euthyroid range. (3)

TEST PRINCIPLE
Sandwich principle. Total duration of assay: **80 minutes.**
 • Sample, Anti-TSH coated microwells and enzyme labeled Anti-TSH are combined.
 • During the incubation, TSH presents in the sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies.
 • After washing, a complex is generated between the solid phase, the TSH within the sample 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 absorbance is proportional to the amount of TSH in the sample.

REAGENTS
Materials provided
•**Coated Microplate**, 8 x 12 strips, 96 wells, pre-coated with mouse monoclonal Anti-TSH.
• **Calibrators**, 6 vials, 1 ml each, ready to use; Concentrations: 0(**A**), 0.5(**B**), 2.5(**C**), 7.5(**D**), 20(**E**) and 40(**F**) µIU/mL.
• **Enzyme Conjugate**, 1 vial, 11 mL of HRP (horseradish peroxidase) labeled mouse monoclonal Anti-TSH in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% 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 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.

• 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 7 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 **25 µL** of **calibrators** or **samples** to each well.
• Add **100 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 6**0 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 µIU/ml 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.

SAMPLE	VALUE (pmol/L)	Absorbance
Calibration A	0	0.015
Calibration B	0.5	0.108
Calibration C	2.5	0.433
Calibration D	7.5	1.106
Calibration E	20	1.877
Calibration F	40	2.828
Control 1	1.10	0.206
Control 2	8.76	1.184
Sample	1.31	0.239

LIMITATIONS – INTERFERENCE

•The assay is unaffected by icterus (bilirubin < 600 μ mol/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.

• There is no high-dose hook effect at TSH concentrations up to 2000 μ IU/mL. In vitro tests were performed on 26 commonly used pharmaceuticals.

• The presence of autoantibodies may induce high molecular weight complexes (macro-TSH) which may cause unexpected high values of TSH.

• 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.

• Serum TSH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.

• A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and thyroxine. Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

• For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

LIMITS AND RANGES

Measuring Range

0.02- 40 μ IU/mL (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 0.02 μ IU/mL. Values below the detection limit are reported as < 0.02 μ IU/mL. Values above the measuring range are reported as > 40 μ IU/mL (or up to 400 μ IU/mL for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit

Lower detection limit: 2 0.01 µIU/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

0.37-5.10 μ IU/mLThese values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 577 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 DiaSino 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	0.057	0.005	8.54	0.00	8.69
Serum 2	0.44	0.024	5.46	0.03	6.74
Serum 3	3.11	0.146	4.71	0.20	6.32
PC universal 1	1.63	0.088	5.38	0.09	5.75
PC universal 2	7.86	0.321	4.09	0.42	5.36

Repeatability = within-run precision

METHOD COMPARISON

A comparison of the TSH assay (y) with the Roche Elecsys TSH (x) using clinical samples gave the following correlations:

Linear regression y = 1.003X -0.629, I = 0.9780

The sample concentrations were between approx. 0 and 38 µIU/mL.

For the monoclonal antibodies used, the following cross-reactivities were found: LH 0.041 %, FSH 0.001 %; hGH and hCG no cross-reactivity.

FUNCTIONAL SENSITIVITY

0.02 µIU/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

REFERENCES

1. Barker, S.B., "Determination of Protein Bound Iodine."
2. *Joumal Biological Chemistry*, 173, 175, (1984).
3. Caldwell, G et al, "A new Strategy for Thyroid Test in the Routine Laboratory Tests." *Lancet*, I, 1117 (1985).
4. Young DS, Pestaner LC, and Gilberman U, "Effects of Drugs on Clinical Laboratory Tests", *Clinical Chemistry*, 21, 3660 (1975)