TSH ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIATSH1	TSH ELISA	96 Tests

Intended Use:
Enzyme Immunoassay for the in vitro quantitative determination of thyroid stimulating hormone in human serum.

Summary and Principle:
Thyroid stimulating hormone (TSH) is an essential hormone for the maintenance of normal thyroid function. The measurement of TSH is considered as the most sensitive indicator of primary and secondary hypothyroidism. It is also useful in differentiating secondary and tertiary hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing hormone which is secreted by the hypothalamus and by direct action of T4 and T3.
The TSH ELISA is based on a one step sandwich method. Microwells are coated with anti-TSH antibody. During the reaction, samples containing TSH and Enzyme Conjugate containing Anti-TSH antibody labelled with HRP are mixed and incubated. TSH present in the sample reacts simultaneously with the coated and the conjugate antibodies resulting in an antibody-antigen-antibody immunocomplex bound to the well surface. After washing, which removes excess antigens and unreacted antibody, Substrate Solution is added which uses the HRP enzyme on the labelled immunocomplexes to catalyse the oxidation of the substrate with the development of a blue coloured product. The reaction is stopped by the addition of Stop Solution which also turns the blue colour to yellow. The intensity of colour measured spectrophotometrically is directly proportional to the concentration of TSH in the sample.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with mouse monoclonal Anti-TSH. The microwells can be broken and used separately. Place unused wells or strips in the plastic sealable bag provided together with the desiccant and store at 2 - 8°C. The wells are stable until expiry at 2 - 8°C if stored as described above.
Calibrators	6x1ml	Ready to use. The exact concentrations (µIU/mL) are provided on the vial labels. Concentrations given on the IFU are subject to change. Once open stable until expiry at 2 - 8°C.
Enzyme Conjugate	1x11ml	1 vial of Horseradish peroxidise (HRP) labelled mouse monoclonal Anti-TSH in Tris-NaCl buffer containing BSA (bovine serum albumin). Contains 0.1% ProClin3000 preservative. Once open, stable until expiry at 2 - 8°C.
Substrate Solution	1x11ml	Ready to use tetramethylbenzidine (TMB). Once open, stable until expiry at 2 - 8°C.
Wash Buffer (40X)	1x25ml	PBS-Tween wash solution. 40X concentrate. Once diluted, stable for two months at ambient temperature (15 - 25°C).
Stop Solution	1x6ml	1 vial sulfuric acid (1 mol/l). Ready to use. Once open, stable until expiry at 2 - 8°C.

IFU, Plate covers, sealable plastic bag.

Materials required but not provided:
Microplate reader with 450nm and 630nm wavelength absorbance capability, microplate washer, incubator, plate shaker, micropipettes, absorbent paper, distilled water.

Precautions and Safety:
• For in vitro diagnostic use only. For professional use only.
• The ELISA assay is time and temperature sensitive. To avoid incorrect results, follow the test procedure steps exactly.
• All products that contain human serum or plasma have been tested and found to be non-reactive for HBsAg, HCV and HIV I/II. But all products should be regarded as potentially biohazardous in use and for disposal.
• Mix the sample in the wells thoroughly by sharply tapping the side of the plate and eliminate air bubbles.
• Conduct assay away from poor ambient conditions such as air containing high concentrations of corrosive gas including sodium hypochlorite acid, alkalis, and acetaldehydes, or containing dust.
• A thorough washing procedure is essential for obtaining accurate results. Use of an automatic plate washer is recommended. Each well must be filled with wash solution but avoid overflowing which may contaminate adjacent wells. After each wash, fully expel all liquid with a sharp flick of the plate. After the final wash cycle, sharply tap the microplate onto absorbent paper to remove all residual wash solution.
• Do not use reagents beyond the labelled expiry date.
• Do not mix or use components from kits with different batch numbers.
• 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 the bottom of the plate is clean and dry
• Ensure there are no bubbles present on the surface of the liquid before reading the plate.
• The timing of substrate and stop solution additions across the plate should be the same for each reagent to eliminate differences in incubation time for the samples.

Specimen Collection:
Collect serum by separation after standard venepuncture technique. Cap and store serum at 15 – 25°C for no more than 8 hours. Stable for 7 days at 2 - 8 °C or for 1 month at -20 °C. Recovery within 90-110% of serum value or slope 0.9-1.1.
Freeze only once.

Storage and Stability:
Store unopened kits at 2 - 8°C at all times. Once kits are opened, place unused wells in the zip-lock aluminium foil pouch along with the desiccant and return to 2 - 8°C, under which wells will remain stable for 2 months, or until labelled expiry date, whichever is earlier. Tightly recap all reagent vials and calibrators and return to 2 - 8°C, under which 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 other unused reagents to 2 - 8°C. When stored under these conditions all kit components will be stable for 2 months or until the labelled expiry date, whichever is earlier.
• 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 stabilised with azide.
• Ensure samples, calibrators, and controls are brought to ambient temperature (15 -25 °C) before use.
• If sediments are present in samples they should be removed by centrifugation as their presence may interfere in the assay. 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 samples are not decayed prior to use.
• Do not use grossly haemolytic, lipemic or turbid samples.
• Note that interfering level 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.
• Mix all reagents through gently inverting prior to use.
• Prepare wash solution concentrate before measurement. Stable for 2 months at ambient temperature.
• Do not use substrate if the reagent is blue in colour
• Do not use reagents which are contaminated or have bacterial growth.

Quality Control:
Each laboratory should run Quality Controls in each assay run covering the assay range at the levels low, normal and elevated for monitoring assay performance. The controls should be treated as unknowns and values determined in every test procedure performed. Results for unknown samples tested are valid if the Quality Control values fall within the assigned concentration ranges for each level.

Procedure:
Reagent preparation:
Bring all samples, calibrators, and controls to room temperature (15 - 25 °C). Mix all reagents through gently inverting prior to use. Prepare Wash Solution by adding the contents of the Wash Buffer Concentrate bottle to 735 ml of distilled water.
If calibrators are supplied lyophilised, reconstitute each vial with 0.5 ml distilled water. Leave to stand for 20 minutes then mix well before use. Store at 2 - 8°C.

STEP 1
Preparation: Remove the number of wells required and assign calibrators, controls and samples to well positions for the assay run. Use only the number of wells required.

STEP 2
Addition of Calibrators: Add 25 µl of calibrators, controls and samples to assigned wells.

STEP 3
Addition of Conjugate: Add 100 µl of Enzyme Conjugate to each well. Shake the microplate gently for 30 seconds to mix.

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

STEP 5
Wash step: 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. 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.

STEP 6
Addition of Substrate: Add 100 µl of Substrate Solution to each well.

STEP 7
Incubation: Incubate at room temperature (15 - 25°C) in the dark for 20 minutes. Do not shake the plate after substrate addition.

STEP 8
Stopping the Reaction: Add 50 µl of Stop Solution to each well and mix gently for 15 – 20 seconds until the well contents change from blue to yellow.

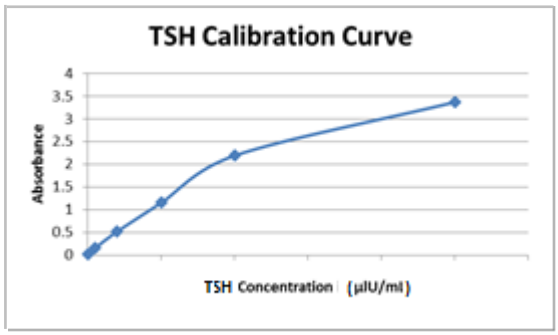
STEP 9
Measurement: Read the absorbance of each well at 450nm (using 620 to 630nm as the reference wavelength if available) in a micro plate reader. The results should be read within 30 minutes of adding the Stop Solution.

Calculation of results:
Record the absorbance obtained from the printout of the microplate reader. Calculate the mean absorbance of any duplicate measurements. Plot the 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.

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

Example:

Sample	Value (µIU/ml)	Absorbance
Calibrator A	0	0.015
Calibrator B	0.5	0.108
Calibrator C	2.5	0.433
Calibrator D	7.5	1.106
Calibrator E	20	1.877
Calibrator F	40	2.826
Control 1	1.10	0.206
Control 2	8.76	1.184
Sample	1.31	0.239



DO NOT USE THE ABOVE CURVE IN LIEU OF THE CALIBRATION.
CALIBRATION CURVES DIFFER DUE TO THE INSTRUMENT USED AND TECHNIQUE ADOPTED.

Limitations – Interferences:

- The assay is unaffected by icterus (bilirubin <600µmol/L or <35mg/dL), haemolysis (Hb <0.559mmol/L or 0.9g/dL), lipemia (Intralipid <1200 mg/dl), and biotin <94nmol/l or <23ng/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 sample 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 µIU/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 wither 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, iodine, 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 µIU/ml (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as <0.02 µIU/ml. Values above the measuring range are reported as >40 µIU/ml (or up to 400 µIU/ml for 10-fold diluted samples).

Lower limits of measurement
Lower detection limit: 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 µIU/ml.
These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 577 healthy test subjects. We have not studied the reference intervals in children, adolescents, and pregnant women.
Each laboratory should determine a reference range pertinent to its own population.

Performance Characteristics:
Representative performance data is given below. Results obtained from individual laboratories may differ.

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

Sample	Mean µIU/ml	Repeatability* SD µIU/ml	Repeatability* CV %	Intermediate Precision SD µIU/ml	Intermediate Precision CV %
Human Serum 1	0.057	0.005	8.54	0.00	8.69
Human Serum 2	0.44	0.024	5.46	0.03	6.74
Human 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: Number of samples measured: 188
Linear regression
y = 1.003x – 0.629
r = 0.978
The sample concentrations were between approx. 0 and 38 µIU/ml.

Analytical specificity:
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 immediate precision CV of 20%.

References:

1. Barker, S.B., "Determination of Protein Bound Iodine."
2. *Journal 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).

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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