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. Once open the wells are stable until expiry at 2 - 8°C.
Calibrators	7x1ml	Ready to use. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABELS. Once open stable until expiry at 2 - 8°C.
Enzyme Conjugate	1x12ml	Horseradish peroxidise conjugated goat Anti-TSH in buffer. Once open, stable until expiry at 2 - 8°C.
Substrate Solution	1x12ml	Ready to use as supplied. Once open, stable until expiry at 2 - 8°C.
Wash Buffer (50X)	1x15ml	PBS-Tween wash solution. Once diluted, stable for two months at ambient temperature.
Stop Solution	1x12ml	Hydrochloric Acid solution (2N). 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 620nm wavelength absorbance capability, microplate washer, micropipettes, absorbent paper, distilled water.

Specimen Collection:
Collect serum by separation after standard venepuncture technique. TSH is stable in serum for up to 7 days at 2 - 8 °C or for 1 month at -20 °C.
If sediments are present in samples they should be removed by centrifugation as their presence may interfere in the assay.
Do not use grossly haemolytic or lipemic samples.
Make sure samples, calibrators, and controls are brought to room temperature (18-25 °C) before measurement.

Storage and Stability:
Store unopened kits at 2 - 8°C at all times. Once kits are opened, place unused wells in the zip-lock plastic bag along with the desiccant and return to 2 - 8°C and tightly recap all reagent vials and return to 2 - 8°C. When stored under these conditions all kit components will be stable until the expiry date.

Precautions and Safety:
The ELISA assay is time and temperature sensitive. To avoid incorrect results, follow the test procedure steps exactly.
For in vitro diagnostic use only by trained professionals only.
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.
A thorough washing procedure is essential for obtaining accurate results. Use of an automatic plate washer is recommended. For each wash cycle leave the plate to soak for one minute. After the last wash cycle invert the plate onto absorbent paper and tap the plate to remove all remaining remnants of wash buffer.
Do not use reagents beyond the labelled expiry date.
Do not mix or use components from kits with different batch numbers.
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.

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.

STEP 2
Addition of Calibrators: Add 50 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 for 30 seconds to mix.

STEP 4
Incubation: Cover the plate with the plate cover and incubate for 60 minutes at room temperature (15 - 25°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.

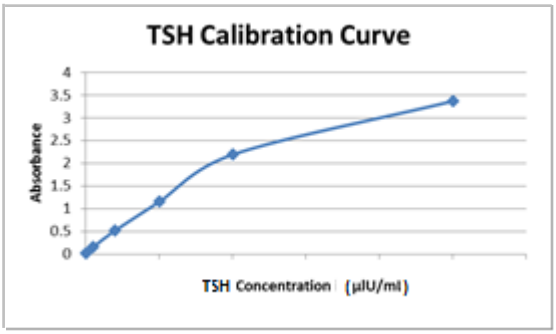
STEP 7
Incubation: Incubate at room temperature (15 - 25°C) in the dark for 20 minutes. Mix gently for 5 seconds.

STEP 8
Stopping the Reaction: Add 100 µl of Stop Solution to each well and mix gently 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 graph below provides an example of a typical calibration curve but cannot be used in place of data generation for each assay.



Performance Characteristics:

Interferences
Enzyme immunoassays potentially demonstrate interference by samples containing rheumatoid factor and antinuclear antibodies, and samples from patients receiving treatments containing mouse monoclonal antibodies. The reagents have been formulated to reduce such interference, but the effects may not be eliminated completely.

Measuring range
0.1 - 50 µIU/ml (defined by the lowest detection limit and the maximum of the master curve).

Expected values
0.39 - 6.16 µIU/ml.
These values correspond to the 2.5th and 97.5th percentiles of results obtained from a total of 139 healthy donor samples. Each laboratory should determine a reference range pertinent to its own population.

Precision
Intra assay Precision:
Two human serum pools were run for 20 replicates. The precision results were:

Pool	Mean	SD	CV%
1	2.21	0.11	4.97
2	10.02	0.52	5.18

Inter assay Precision:
Two human serum pools were run in replicates of 2 across 20 days. The precision results were:

Pool	Mean	SD	CV%
1	1.96	0.11	5.46
2	10.29	0.72	6.80

Analytical specificity
Some potentially interfering antigens were spiked into samples and tested in the TSH ELISA. The following cross-reactivities were observed: LH 0.041 %, FSH 0.001 %; hGH and hCG no cross-reactivity.

Quality Control
Each laboratory should run Quality Controls in each assay run covering the assay range. Results for unknown samples tested are valid if the Quality Control values fall within the assigned concentration ranges for each level.

Limitations:

The result from this test should not be used as the sole criteria for diagnosis, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.

References:

1. Soos M and Siddle K. J Immunol Methods 1982; 51: 57 – 68.
2. Wada HG, Danisch RJ and Baxter SR. Clin Chem 1982; 28: 1862-1866.
3. Uotila M, Ruoslahti E and Engvall E. J Immunol Methods 1981; 42: 11-15.
4. Burger HG and Patel YC. Thyrotropin releasing hormone TSH. Clinic Endocrinol and Metab 1977; 6: 831
5. Snyder PJ and Utiger RD. J Clin Endocrinol Metab 1972; 34: 380-385.

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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