# TurboTSH [I-125] IRMA KIT

(REF: RK-1CT1)

The $^{125}$I-hTSH IRMA system provides direct quantitative *in vitro* determination of human Thyroid Stimulating Hormone (hTSH) in human serum. hTSH can be assayed in the range of 0-100 µIU/mL using 100 µL serum samples.

## Introduction

The Thyroid Stimulating Hormone (thyrotropin or TSH) is a glycoprotein with a molecular weight of 28000, secreted by the adenohypophysis. Like other glycoprotein hormones (FSH, LH and HCG), TSH contains two different subunits, an α- and a ß-chain, linked by noncovalent bounds. The primary structure of α subunits of hTSH and of the gonadotrophins is the same, whilst their ß subunits are different. The ß subunits are responsible for the immunological and biological specificity of these hormones.

The synthesis and the release of hTSH are controlled by the circulatory level of thyroid hormones; triiodothyronine (T3) and thyroxin (T4) and by the hypothalamic Thyrotropin-Releasing Hormone (TRH). Thyroid hormones regulate the secretion of hTSH by a negative feed-back mechanism. An elevation of T3 or T4 will suppress, and their fall will, in turn, increase the level of hTSH in serum. The increased concentration of hTSH in the serum is the earliest and best indicator of primary hypothyroidism.

The determination of hTSH by immunoassay methods plays a crucial role in the diagnosis of thyroid disorders and in the evaluation of the functional integrity of the hypothalamic-pituitary axis.

## Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The $^{125}$I labeled signal-antibody binds to an epitope of the hTSH molecule spatially different from that recognized by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During incubation the immuno-complex is immobilized to the reactive surface of streptavidin-coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hTSH, the unknown concentration of hTSH in patient samples can be determined.

## Contents of the kit

**1**. 1 bottle of TRACER, Ready to use. 12 mL per vial, containing < 980 kBq $^{125}$I-signal and capture antibody in buffer with red dye and 0.1 % $NaN_3$.

**2.** 8 vials of STANDARDS (8 x 1.0 mL), containing 0 ($S_0$), 0.06 ($S_{0.06}$), 0.15 ($S_{0.15}$), 0.6 ($S_{0.6}$), 2.5 ($S_{2.5}$), 15 ($S_{15}$), 50 ($S_{50}$) and 100 ($S_{100}$) µIU/mL hTSH (WHO 3rd IRP 81/565) in horse serum with 0.1% $NaN_3$.

**3.** 2 vials of CONTROL SERA (2 x 1.0 mL). Low (CI), and high (CII). Human serum containing 0.1% $NaN_3$. The concentrations of controls are specified in the quality certificate enclosed.

**4.** 2 boxes of COATED TUBES, ready to use.

2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

Quality certificate

Pack leaflet

## Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100 and 2000 µL), distilled water, vortex mixer, shaker, plastic foil, adsorbent tissue, gamma counter.

***Recommended tools and equipment***

Repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-mL pipette).

## Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep-frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens.

## Storage of reagents

Store the reagents between 2-8°C after opening. At this temperature each reagent is stable until the expiration date of the kit. The actual expiry date is given on the package label and in the quality certificate.

CAUTION!

Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

## Assay procedure

***Note:*** *When the KIT has less than 3 weeks to expiration or when using an automated procedure,* ***the standard 0.06 µIU/mL should be omitted****.*

1. Label coated tubes in duplicate for each standard, control serum & samples.
2. Pipette 100 µL of standards, control & samples into the properly labelled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
3. Pipette 100 µL of tracer into each tube. (Optionally, set aside 2 uncoated tubes for total counts.)
4. Seal all tubes with a plastic foil. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed so that liquid is constantly rotating or swirling in each tube. (min. 600 rpm recommended).
5. Incubate tubes for 30 minutes, shaking at room temperature.
6. Add 2.0 mL of distilled water to each tube. Decant or aspirate the content of tubes by inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
7. Return the tube-rack to an upright position, and repeat step-6 two more times.
8. Count each tube for at least 60 seconds in a gamma counter.
9. Calculate the hTSH concentrations of the samples as described in calculation of results or use special software.

**Table 1. Assay protocol, Pipetting Guide**

(volumes in µL)

| Tubes | Total | Standard | Control | Sample |
|---|---|---|---|---|
| Standard | | **100** | | |
| Control | | | **100** | |
| Sample | | | | **100** |
| Tracer | **(100)** | **100** | **100** | **100** |
| Shake for 30 minutes at room temperature. | | | | |
| Wash and decant three times with 2 mL of distilled water | | | | |
| Count radioactivity (60 sec/tube). | | | | |
| Calculate the results. | | | | |

## Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (cpm) for each pair of assay tubes. Calculate the normalized percent binding for each standard, control & sample respectively by using the following equation:

$$B/T\ (\%) = \frac{Sx/C/Mx/\ (cpm) - S0(cpm)}{T\ (cpm)} \times 100$$

Using logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of hTSH.

Determine the hTSH concentration of the controls & unknown samples by interpolation from the standard curve.

Automated data processing systems are also applicable.

**Table 2. Typical assay data**

| Tubes | Mean cpm | B/T% |
|---|---|---|
| Total | 375 646 | |
| S0 (NSB) | 160 | |
| S0.06 | 275 | 0.03 |
| S0.15 | 462 | 0.08 |
| S0.6 | 1 233 | 0.29 |
| S2.5 | 4 899 | 1.26 |
| S15 | 26 309 | 6.96 |
| S50 | 79 850 | 21.21 |
| S100 (Bmax) | 150 083 | 39.91 |
| CI | 1 991 | 0.49 |
| CII | 31 386 | 8.31 |

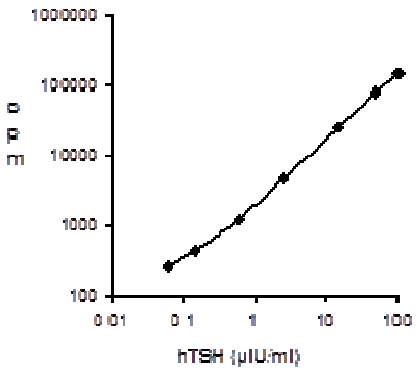


Figure 1.
A typical standard curve
*(Do not use to calculate sample values!)*

## Performance characteristics

### Sensitivity

The analytical sensitivity is **0.009 µIU/mL**, obtained by assaying 31 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17.

**LoB = 0.015 µIU/mL** determined as the highest measurement result that is likely to be observed (with a stated probability [5%]) for a blank sample.

**LoD = 0.036 µIU/mL** determined with proportions of false positives (α) less than 5 % and false negatives (ß) less than 5 %, based on 116 determinations, with 4 blanks and 4 low level samples.

**LoQ = 0.070 µIU/mL**, as graphically determined from the precision profile curve.
*LoQ = functional sensitivity*

### Precision and reproducibility

Five human serum pools were assayed in 20 replicates to determine intra-assay precision.
Intra-assay precision table:

| Sample ID | Mean µIU/mL | Intra-assay CV% |
|---|---|---|
| Pool 1 | 1.91 | 1.85 |
| Pool 2 | 2.37 | 2.41 |
| Pool 3 | 8.39 | 1.19 |
| Pool 4 | 19.84 | 1.68 |
| Pool 5 | 45.86 | 1.21 |

To determine inter-assay precision 7 human serum pools were measured in duplicates in 20 independent assays by 6 operators using different kit batches. Values obtained are shown below.
Inter-assay precision table:

| Sample ID | Mean µIU/mL | Inter-assay CV% |
|---|---|---|
| Pool 1 | 0.076 | 19.3 |
| Pool 2 | 0.152 | 11.5 |
| Pool 3 | 2.149 | 3.0 |
| Pool 4 | 2.249 | 3.5 |
| Pool 5 | 7.531 | 2.6 |
| Pool 6 | 18.939 | 2.7 |
| Pool 7 | 38.489 | 2.0 |

### Linearity – dilution test

Four individual human serum samples were serially diluted with zero standard. They were measured according to kit protocol. Mean recovery after zero standard dilution was 95.36 %.

The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$Y = 1.0361x - 0.7669 \quad R^2 = 0.9994 \quad n = 16$

### Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of TSH. The average per cent recovery for 4 serum samples spiked with TSH at 3 levels each was 102.28%, with a range of 99% to 108%.

### Hook effect

There is no high dose hook effect up to an hTSH concentration of 8000 µIU/mL.

### Specificity

Cross-reaction is undetectably low in the physiological ranges of LH, FSH and hCG.

### Reference Interval

It is recommended that each laboratory establish its own reference intervals. The expected values presented here are based on testing of apparently healthy blood donors. Samples were measured in duplicates.
The reference range for presumably healthy individuals is **0.30 – 4.00 µIU/mL.**

## Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Note for shaking step:** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Decantation:** Decantation is the most critical step of the assay procedure. Pay a special attention not to contaminate the outer surface of tubes, when turning the test tube-rack upside down. Even a small contamination may introduce a high, unidentified background resulting in a substantial over-estimation of concentration. The error associated may become particularly high in the low range of concentration, which is of vital importance for the reliable determination of subnormal TSH-values. For the same reason, regular checking of the instrument background is inevitable. This is particularly important, when multi-channel counters are used. Make ensure that background values and variation between individual channels are within the range of acceptance as specified in counter's service book.

**Additional information:** Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

## Limitations

- The non-respect of the instructions in this insert may affect results significantly.
- Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other diagnostic procedures.
- Specimens from patients who have received mouse immunoglobulin for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Serum from such individuals may produce erroneous results.

## Precaution

### Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

### Biohazard

*Human blood products* used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1, 2), Hepatitis-C antibody (anti-HCV), Hepatitis B surface Antigen (HBsAg) and Treponema Antibody.Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials.*
*All animal products* and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials.*

### Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 22 mg.

## Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 67 days from availability.

| | | | |
|---|---|---|---|
| | Use by | CONTROL | Control |
| LOT | Batch code | CAL | Standard |
| | Caution, consult accompanying documents | CT | Coated tube |
| | Biological risk | TRAC | Tracer |
| | Consult operating instructions | REF | Catalogue number |
| IVD | In vitro diagnostic medical device | 2 8°C | Temperature limitation Store between 2-8ºC |
| | Manufacturer | | Radioactive Material |

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