TurboTSH [I-125] IRMA KIT  
(REF: RK-1CTS)

The 125I-labeled 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 β-chain, linked by noncovalent bonds. The primary structure of the subunits of hTSH and of the gonadotrophins is the same, whilst their β subunits are different. The β subunits are responsible for the immunological and biological specificity of these hormones.

The synthesis and the release of TSH 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 TSH by a negative feedback mechanism. An elevation of T3 or T4 will suppress, and their fall will, in turn, increase the level of TSH in serum. The increased concentration of TSH in the serum is the earliest and best indicator of primary hypothyroidism.

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

Principle of method
The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system. The 125I 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. 55 mL per vial, containing < 4.5 MBq 125I-signal and capture antibody in buffer with red dye and 0.1 % NaN3.

2. 8 vials of STANDARDS (8 x 1.5 mL), containing 0 (S0), 0.06 (S0.06), 0.15 (S0.15), 0.6 (S0.6), 2.5 (S2.5), 15 (S15), 50 (S50) and 100 (S100) µIU/mL hTSH (WHO 3rd IRP 81/565) in horse serum with 0.1% NaN3.

3. 2 x 2 vials of CONTROL SERA (1.5 mL/vial), Low (CL), and high (CH). Human serum containing 0.1% NaN3. The concentrations of controls are specified in the quality certificate enclosed.

4. 10 boxes of COATED TUBES, ready to use. 10 x 50 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 200 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 2-8°C if the assay is carried out within 24 hours, otherwise aliquots should be prepared

Store the reagents between 2-8°C after opening. When the KIT has less than 3 weeks to expiration or when using an automated data processing systems are also applicable. Automated data processing systems are also applicable.

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:

\[
B/T (%) = \frac{S_x/C_x M_x}{(cpm) - S_0 (cpm)} \times 100
\]

where \( S_x \) is the cpm of standard, \( C_x \) is the cpm of control, \( M_x \) is the cpm of sample, and \( S_0 \) is the cpm of negative control.

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.

Table 1. Assay protocol, Pipetting Guide (volumes in µL)

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Total</th>
<th>Standard</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracer</td>
<td>(100)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Wash and decant three times with 2 mL of distilled water

Count radioactivity (60 sec/tube).

Calculate the results.

Table 2. Typical assay data

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Mean cpm</th>
<th>B/T%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>375 646</td>
<td></td>
</tr>
<tr>
<td>S0 (NSB)</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>S0.06</td>
<td>275</td>
<td>0.03</td>
</tr>
<tr>
<td>S0.15</td>
<td>462</td>
<td>0.08</td>
</tr>
<tr>
<td>S0.6</td>
<td>1 233</td>
<td>0.29</td>
</tr>
<tr>
<td>S2.5</td>
<td>4 899</td>
<td>1.26</td>
</tr>
<tr>
<td>S15</td>
<td>26 309</td>
<td>6.96</td>
</tr>
<tr>
<td>S50</td>
<td>79 850</td>
<td>21.21</td>
</tr>
<tr>
<td>S100 (Bmax)</td>
<td>150 083</td>
<td>39.91</td>
</tr>
<tr>
<td>CL</td>
<td>1 991</td>
<td>0.49</td>
</tr>
<tr>
<td>CH</td>
<td>31 386</td>
<td>8.31</td>
</tr>
</tbody>
</table>
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 (a) less than 5 % and false negatives (b) 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:

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mean µIU/mL</th>
<th>Intra-assay CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 1</td>
<td>1.91</td>
<td>1.85</td>
</tr>
<tr>
<td>Pool 2</td>
<td>2.37</td>
<td>2.41</td>
</tr>
<tr>
<td>Pool 3</td>
<td>8.39</td>
<td>1.19</td>
</tr>
<tr>
<td>Pool 4</td>
<td>19.84</td>
<td>1.68</td>
</tr>
<tr>
<td>Pool 5</td>
<td>45.86</td>
<td>1.21</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Mean µIU/mL</th>
<th>Inter-assay CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool 1</td>
<td>0.076</td>
<td>19.3</td>
</tr>
<tr>
<td>Pool 2</td>
<td>0.152</td>
<td>11.5</td>
</tr>
<tr>
<td>Pool 3</td>
<td>2.149</td>
<td>3.0</td>
</tr>
<tr>
<td>Pool 4</td>
<td>2.249</td>
<td>3.5</td>
</tr>
<tr>
<td>Pool 5</td>
<td>7.531</td>
<td>2.6</td>
</tr>
<tr>
<td>Pool 6</td>
<td>18.939</td>
<td>2.7</td>
</tr>
<tr>
<td>Pool 7</td>
<td>38.489</td>
<td>2.0</td>
</tr>
</tbody>
</table>

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:

\[ Y = 1.036X - 0.7669 \quad R^2 = 0.9994 \quad n = 16 \]

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 up effect to an ITSH 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 firmly tightened 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.

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 123 mg.

Storage and shelf life

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

INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: (36-1)392-2577, Fax: (36-1)395-9247

Website: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu