

Contents of the kit

1. 1 vial TRACER, lyophilized, reconstitution with 12.5 ml assay buffer, containing ~ 82 kBq, 2.2 µCi [\(^{125}\text{I}\)]TSH. The final solution contains [\(^{125}\text{I}\)]TSH in 0.025 M phosphate buffer pH7.5 containing 0.1% (w/v) sodium azide. Store at 2-8°C.
2. 1 vial STANDARD, lyophilized, reconstitution with assay buffer, volume stated on the vial label. The final solution contains rTSH at a concentration of 64 ng/ml. Store at 2-8°C.
3. 1 vial ANTI SERUM, lyophilized, reconstitution with 12.5 ml assay buffer. The final solution contains rabbit anti-rTSH serum in 0.025 M phosphate buffer pH7.5 containing 0.1% (w/v) sodium azide. Store at 2-8°C.
4. 1 vial ASSAY BUFFER (10.0 ml), dilution to 100 ml. On dilution this will give 0.025 M phosphate buffer, pH7.5, containing 0.1% (w/v) sodium azide. Store at 2-8°C.
5. 1 vial SEPARATING SECOND ANTIBODY REAGENT (58 ml), ready for use, containing donkey anti-rabbit serum coated on to magnetisable polymer particles with sodium azide, colour-coded, blue-green. Store at 2-8°C.

Pack leaflet

Materials, tools and equipment required

 Pipettet or pipetting equipment with disposable tips (100µl, 200µl, 400µl, 500µl, 2.0ml and 12.5ml); disposable polypropylene or polystyrene tubes (12 x 75 mm); refrigerator; glass measuring cylinder (100ml); test tube rack; vortex mixer; plastic foil; separators, comprising magnetic base and assay rack - these are for use in the magnetic separation protocol; adsorbent tissue; gamma counter.

Specimen collection, storage

This section is provided for guidance only. It remains the investigator’s responsibility to validate the chosen sample collection technique.

Blood samples

It is advised that if measurements are to be made on plasma samples, blood should be collected into tubes containing heparin (<20 units per ml of blood). Blood should be centrifuged immediately to remove cells and the plasma stored below -15°C prior to analysis. Serum samples can also be assayed with this kit. Samples may need to be diluted prior to assay depending on the expected concentration. Assay buffer remaining after reconstitution of assay components may be used for this.

Tissue samples

Pituitary glands should be frozen immediately after removal and stored at -15°C to -30°C until required. Prior to analysis, glands should be homogenised or sonicated in phosphate buffered saline solution. Samples may need to be diluted depending on the expected concentration. Assay buffer remaining after reconstitution of assay components may be used for this.

Preparation of reagents, storage

Storage: see Contents of the kit. At these temperatures each reagent is stable until expiry date. The actual expiry date is given on the package label and on the quality certificate.

Preparation: Equilibrate all reagents and samples to room temperature prior to use. Assay buffer: Warm the bottle containing assay buffer concentrate to 40°C or until the gel-like material melts. Temperatures above 60°C should be avoided. Transfer the contents of the bottle, with washings, to a 100 ml measuring cylinder and dilute to 100 ml with distilled or deionised water. Mix well. Assay buffer is used to reconstitute all other components. The other components (except Separating reagent): Carefully add the required volume of assay buffer and replace stopper. Mix the contents of the bottles by inversion and swirling, taking care to avoid foaming.

Preparation of working standards

1. Label 6 polystyrene or polypolyethylene tubes 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2.
2. Pipette 500 µl of assay buffer into all tubes.
3. Into the 3.2 tube pipette 500 µl of stock standard (64 ng/ml) and vortex thoroughly.
4. Transfer 500 µl from the 3.2 tube to the 1.6 tube and vortex thoroughly.
5. Repeat this doubling dilution successively with the remaining tubes.
6. 100 µl aliquots from each serial dilution give rise to 6 standard levels of rTSH ranging from 0.1 ng to 3.2 ng/tube. Note: One hundred microlitres (100 µl) of the reconstituted stock standard provided serves as the top standard (64.4 ng/tube). Working standards should be freshly prepared before each assay, and not re-used.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Equilibrate all reagents to room temperature.
2. Prepare reagents and assay standards as described in the previous section.
3. Label 12 x 75 mm disposable tubes in duplicate for total count (TC), non-specific binding (NSB), zero standard (Bo), standards and samples.
4. Pipette 200 µl assay buffer into NSB tubes and 100 µl assay buffer into Bo tubes.
5. Starting with the most dilute, pipette 100 µl of each standard (S1-7) into the appropriately labelled tubes.
6. Pipette 100 µl unknown sample (M) directly into appropriately labelled tubes.
7. Pipette 100 µl antiserum into all tubes except NSB and TC.
8. Pipette 100 µl of tracer into all tubes. The TC tubes should be stoppered and put aside for counting.
9. Vortex mix all tubes thoroughly. Cover the tubes, and incubate overnight (16-24 hours) at room temperature (15-30°C).
10. Gently shake and swirl the bottle containing separating second antibody reagent (blue-green) to ensure a homogeneous suspension. Add 400 µl into each tube except the TC. Vortex mix all tubes thoroughly and incubate at room temperature for 10 minutes.
11. Separate the antibody bound fraction using either magnetic separation or centrifugation as described below.

**Magnetic separation**

Attach the rack on the magnetic separator base and ensure that all tubes are in contact with the base plate. Leave for 15 minutes. After separation, do not remove the rack from the separator base. Pour off and discard the supernatant. Keeping the separator inverted, place the tubes on a pad of absorbent tissues and allow to drain for 5 minutes.

**Centrifugation**

Centrifuge all tubes at 4°C for 10 minutes at 1500 xg or greater. After centrifugation, place the tubes carefully into suitable decantation racks, then pour off and discard the supernatant. Keeping the separator inverted, place the tubes on a pad of absorbent tissues and allow to drain for 5 minutes.

On completion of either magnetic or centrifugal separation, firmly blot the edges of the inverted tubes on the tissue pad to remove any adhering liquid. Do not re-invert the tubes once they have been turned upright.

12. Determine the radioactivity present in each tube by counting for at least 60 seconds in a gamma scintillation counter.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>TC</th>
<th>NSB</th>
<th>Bo</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>-</td>
<td>200</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Anti-serum</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tracer</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Vortex mix and incubate for 16-24 hours at room temperature (15-30°C).

**Calculation of results**

Calculate the average count per minute (CPM) for each pair of assay tubes. Calculate the percent NSB/TC using the following equation:

\[
\frac{\text{NSB/TC}}{\text{TC}} = \left( \frac{\text{NSB (cpm)}}{\text{TC (cpm)}} \right) \times 100
\]

If the counter background is high, it should be subtracted from all counts.

Users may wish to subtract the average NSB cpm from all tubes except TC. If so the appropriate correction should be made. Calculate the percent Bo/TC using the following equation:

\[
\frac{\text{Bo/TC}}{\text{TC}} = \left( \frac{\text{Bo (cpm)} - \text{NSB (cpm)}}{\text{TC (cpm)}} \right) \times 100
\]

Calculate the percent bound for each standard and sample using the following equation:

\[
\frac{\text{S1-7-M (cpm)} - \text{NSB (cpm)}}{\text{Bo (cpm)} - \text{NSB (cpm)}} \times 100
\]

A standard curve can be generated by plotting the percent B/Bo as a function of the log rTSH concentration. Plot B/Bo (%) (y-axis) against concentration ng standard per tube (x-axis). The concentration (ng per tube) value of the samples can be read directly from the graph (see Figure 1).

<table>
<thead>
<tr>
<th>Tube</th>
<th>Conc. ng/tube</th>
<th>Mean counts (cpm)</th>
<th>B/TC (%)</th>
<th>B/Bo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>-</td>
<td>27243</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NSB</td>
<td>-</td>
<td>643</td>
<td>2.4</td>
<td>-</td>
</tr>
<tr>
<td>Bo</td>
<td>-</td>
<td>13758</td>
<td>48.1</td>
<td>-</td>
</tr>
<tr>
<td>S1</td>
<td>0.1</td>
<td>12515</td>
<td>90.5</td>
<td>-</td>
</tr>
<tr>
<td>S2</td>
<td>0.2</td>
<td>11250</td>
<td>80.9</td>
<td>-</td>
</tr>
<tr>
<td>S3</td>
<td>0.4</td>
<td>9541</td>
<td>67.8</td>
<td>-</td>
</tr>
<tr>
<td>S4</td>
<td>0.8</td>
<td>7061</td>
<td>48.9</td>
<td>-</td>
</tr>
<tr>
<td>S5</td>
<td>1.6</td>
<td>5059</td>
<td>33.7</td>
<td>-</td>
</tr>
<tr>
<td>S6</td>
<td>3.2</td>
<td>3511</td>
<td>21.9</td>
<td>-</td>
</tr>
<tr>
<td>S7</td>
<td>6.4</td>
<td>2260</td>
<td>12.3</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: The counts were obtained using new tracer. The counts will decline in line with the age of the tracer.

A decrease in Bo values will be observed during the shelf-life of this product. However, this drop in binding does not in our experience affect the curve parameters.

**Characterization of assay**

**Stability**

The components of this assay system will have a shelf-life of at least 2 weeks from the date of despatch. Upon arrival, all components should be stored at 2–8°C where they are stable until the expiry date printed on the pack label.

**Non-specific binding**

The non-specific binding (NSB) defined as the proportion of tracer bound in the absence of antibody was determined to be <5%.

**Sensitivity**

The sensitivity, defined as the amount of rTSH needed to reduce zero dose binding by two standard deviations was 0.05 ng/tube.
Speciﬁcity
The cross-reactivity, as determined by the concentration giving 50% B/Bo with a number of related compounds is shown below.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat TSH</td>
<td>100</td>
</tr>
<tr>
<td>rat TSH (NIH-RP2)</td>
<td>103</td>
</tr>
<tr>
<td>rat GH (NIH-RP2)</td>
<td>0.32</td>
</tr>
<tr>
<td>rat FSH</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>rat LH (NIH-RP3)</td>
<td>&lt;0.19</td>
</tr>
<tr>
<td>rat ACTH</td>
<td>&lt;0.008</td>
</tr>
</tbody>
</table>

Reproducibility
The intra-assay precision for duplicate determinations was calculated by repeatedly measuring an unknown in the assay. The result obtained was 0.83 ± 0.04 ng, CV = 4.8%. The between assay precision was assessed by repeated measurement of the same samples in successive assays using different batches of reagents and different operators. These results are shown below:

Dilutions of unextracted plasma and serum gave good parallelism with the standard curve (results from 2 experiments).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of replicates</th>
<th>Mean ± SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>0.37±0.06</td>
<td>16.2</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>0.94±0.08</td>
<td>8.5</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>3.35±0.50</td>
<td>14.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample volume (µl)</th>
<th>Measured conc. (ng/tube)</th>
<th>Calculated conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.18</td>
<td>3.60</td>
</tr>
<tr>
<td>75</td>
<td>0.31</td>
<td>4.13</td>
</tr>
<tr>
<td>100</td>
<td>0.50</td>
<td>5.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample volume (µl)</th>
<th>Measured conc. (ng/tube)</th>
<th>Calculated conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.40</td>
<td>8.00</td>
</tr>
<tr>
<td>75</td>
<td>0.62</td>
<td>8.27</td>
</tr>
<tr>
<td>100</td>
<td>0.87</td>
<td>8.70</td>
</tr>
</tbody>
</table>

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

Precautions
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.

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 158 mg.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

Safety data sheet
Product name:
Sodium azide
CAS No. 26628-22-8
R: 22-32 Toxic if swallowed. Contact with acids liberates very toxic gas. S: (1/2)-28-45 (Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Composition:
Sodium azide solution.

Hazards identification:
Toxic if swallowed, inhaled, or absorbed through skin. May cause eye and skin irritation.

First aid measures:
In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.

Fire fighting measures:
Dry chemical powder. Do not use water.

Accidental release:
Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick-up is complete.

Handling and storage:
Wear suitable protective clothing including overalls, safety glasses and gloves. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling.

Personal protection:
See above instructions for handling and storage.

Physical and chemical properties:

Stability and reactivity:
Avoid contact with metals and acid chlorides. This yields a very toxic gas.

Toxicological information:
LD50: 27 mg/kg oral, rat LD50: 20 mg/kg skin, rabbit

Ecological information:
Not applicable

Disposal consideration:
Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.

Note: Inorganic azides will react with lead and copper plumbing fixtures to give explosive residues. Disposal of significant quantities of azides via such plumbing is not recommended.

Transport information:
No special considerations applicable.

Regulatory information:
The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

Use by: AS Antiserum
LOT: CAL Standard
Caution, consult accompanying antibodies: SAR Separating second antibody reagent
Biological risk: TRAC Tracer
Consult operating instructions: BU6 Assay buffer
Manufacturer: TEMP Temperature limitation
REF: Store between 2-8°C

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

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