1. Intended use

For IN VITRO determination of serum and plasma rat thyrotropin (rTSH).

2. Principle of the method

The rat TSH assay is based on the competition between the TSH of the rat sample and a 125I-labelled rat TSH tracer for binding to a highly specific rabbit polyclonal antibody (Ab) according to the following equation:

\[
\text{rTSH} + \text{Ab} \leftrightarrow \text{rTSH-Ab}
\]

Since the concentrations of 125I-rTSH and Ab are constant, the advancing state of the equation depends on the concentration of rTSH in the sample. After incubation, separation of bound from free is achieved by a second antibody (mouse monoclonal antibody anti rabbit Ig) coupled to magnetic particles. The radioactive bound fraction is precipitated by centrifugation or magnetic separation and counted in a gamma counter.

Rat sample concentrations are read from a calibration curve and the results are expressed in ng/ml.

3. Warnings and precautions

For in vitro use

It must be handled by specialized staff.

Good laboratory and safety practices are advisable.

**Warning:**

This kit contains 125I emitting X and γ ionizing rays.

This radioactive material may be received, acquired, possessed and used only by persons in clinical or hospital laboratories who are authorized by competent authorities and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom , to human beings or animals. Its receipt, acquisition, possession, use, transfer, the waste disposal and the people protection are subject to the State and local regulations.

Use impermeable gloves and appropriate protection clothes.

**Warning:** Some components contain sodium azide (<1g/l). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide building up.

**Warning:** Animal origin materials are also used in this kit, these are provided with sanitary certificate. However, no known test can guarantee that such material does not contain any infectious agents. These products must be considered as potentially infectious and handled with care.

4. Reagents, preparation and storage

All reagents are ready for use, except the tracer, calibrators (0-5) and control.

Stored at 2-8°C, the material can be used up to the expiration date printed on each label.

Before use, reconstitute the content of the calibrator 0 with 4 ml of deionized water and the content of the other calibrators (1-5) and control wth 1 ml of deionized water. Mix gently to avoid foaming. Wait at least 15 minutes after solubilization before dispensing. If not used immediately after reconstitution, the solutions are stable for two weeks at 2-8°C or for longer period if stored at -20°C.

Before use, reconstitute the content of the tracer with 11 ml of reconstitution buffer (BUF). Mix gently to avoid foaming. Wait at least 15 minutes after solubilization before dispensing. After reconstitution, the tracer solution is stable at 2-8°C until the expiration date printed on each label.

After use, close all reagents vials and bottles and replace these at 2-8°C or -20°C.

4.1. RAT TSH 125I

1 vial (11 ml) 125I-labelled rat TSH lyophilized in buffer with a stablizer and a preservative (sodium azide < 1 g/l). The vial contains a maximum of 170 KBq (4.6 µCi) at the iodination date.

4.2. Ab Rat TSH

1 vial (11 ml, blue) highly specific rabbit antiserum anti native rat TSH diluted in buffer with a stablizer, a preservative (NaN3 < 1 g/l) and a blue dye.

4.3. CAL 0

1 vial lyophilized horse serum containing preservatives (NaN3 < 1 g/l) and calibrated against sera from hypox rats. The CAL 0 must also be used to dilute samples with concentrations above the higher calibrator (CAL 5).

4.4. CAL 1-5

5 vials lyophilized rat TSH supplied in horse serum containing preservatives (NaN3 < 1 g/l).

The concentrations of the different calibrators are printed on the vial labels and expressed in ng/ml of highly purified rTSH (purity : > 98 % m/m)

Seeing the lack of international references for rTSH we have established a QC procedure which guarantees batch to batch reproducibility for standard curve calibration.

4.5. CONTROL

1 vial lyophilized rat TSH supplied in horse serum containing preservatives (NaN3 < 1 g/l). The control has to be assayed along with the samples and the result compared to the range printed on the vial label.

4.6. BUF

1 vial (15 ml) of a specific buffer containing a stabiizer, a preservative (sodium azide < 1 g/l) and a red dye. This buffer must be used to reconstitute the tracer.

5. Material required but not provided

- bench surfaces protected by absorbent paper to reduce the effects of radioactive spillage.
- waste disposal containers appropriately labelled and designed as suitable for solid or liquid radioactive materials and biological materials.
- manual or automated precision micropipettes with single use tips for dispensing samples or reagents without cross-contamination.
- repeater pipettes (Eppendorf type)
- Incubator or water bath
- vacuum pump connected through a trap for aspiration
- a centrifuge or magnetic plate
- a vortex mixer
- a calibrated gamma scintillation counter
- appropriate graph paper for plotting the results.

6. Methodology

6.1. Collection and handling of serum or plasma samples

Blood samples may be collected either into dry tubes or in the presence of anti-coagulant (EDTA or heparin).

After separation from the red blood cells, plasma or serum samples may be assayed immediately, within 24 hours if stored at 2-8°C or later, after periods as long as several months, if stored at -20°C. Repeated freezing and thawing must be avoided.

**Important Note:** A sensitive assay can be achieved by using a sample volume of 50 µl instead 25 µl. Please refer to point 10.2 to analyze the sensitivity offered by each method.

6.2. Assay procedure

Do not mix reagents of different lots.

Bring the different components of the kit to room temperature prior to use. Perform the assay in duplicates. Calibrators, control and samples must be assayed at the same time. Follow strictly the different steps of the procedure and use interchangeable tips.

Label the tubes for “(Total count)”, NSB, calibrators, control and samples.

1. NSB

Pipette 25 µl (or 50 µl) of calibrator 0 and 100 µl of distilled water into the corresponding tubes.

2. Calibrators

Pipette 25 µl (or 50 µl) of each calibrator into the corresponding tubes.

3. Samples and control

Pipette 25 µl (or 50 µl) of each sample and control into the corresponding tubes.

4. Add 100 µl of antiserum (Ab Rat TSH) to each tube except NSB and « Total count » tubes Mix all tubes with a vortex mixer.

5. Incubate for 3 hours at 37°C.

6. Add 100 µl of Rat TSH 125I tracer to each tube. « Total count » tubes do not participate to the following steps.

7. Incubate overnight at room temperature.

8. Mix the magnetic particles (SORB Ab Fc) with a vortex mixer and add 200 µl to each tube except “Total Count tubes”.

9. Mix all tubes with a vortex mixer and incubate for 1 hour at room temperature without further mixing.
10.2. Analytical sensitivity

Concentrations were computed using a Logit-Log unweighted regression fit. The sample to background ratio was calculated as follows:

\[ \frac{B}{B_0} = \frac{[\text{cal or smp cpm}] / [B_0 (\text{cal 0}) \text{ cpm}]}{100} \]

Draw the standard curve on semilogarithmic paper by plotting the ratio \( \frac{B}{B_0} \) % versus its respective concentration expressed in ng/ml (logarithmic scale).

Rat TSH concentrations in samples may be read directly from the standard curve.

6.4. Example of a typical assay

<table>
<thead>
<tr>
<th>Tube</th>
<th>Classical method (25 µl)</th>
<th>Sensitive method (50 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contents (ng/ml)</td>
<td>Mean cpm</td>
<td>B/00 (%)</td>
</tr>
<tr>
<td>Total counts</td>
<td>33178</td>
<td>32355</td>
</tr>
<tr>
<td>NSB</td>
<td>915</td>
<td>681</td>
</tr>
<tr>
<td>CAL 0</td>
<td>9430</td>
<td>100.0</td>
</tr>
<tr>
<td>CAL 1</td>
<td>8721</td>
<td>91.7</td>
</tr>
<tr>
<td>CAL 2</td>
<td>7826</td>
<td>81.2</td>
</tr>
<tr>
<td>CAL 3</td>
<td>7287</td>
<td>74.8</td>
</tr>
<tr>
<td>CAL 4</td>
<td>5918</td>
<td>56.8</td>
</tr>
<tr>
<td>CAL 5</td>
<td>4062</td>
<td>37.0</td>
</tr>
<tr>
<td>CONTROL</td>
<td>4.0 – 7.0</td>
<td>7349</td>
</tr>
<tr>
<td>Sample 1</td>
<td>6123</td>
<td>61.2</td>
</tr>
<tr>
<td>Sample 2</td>
<td>8195</td>
<td>85.5</td>
</tr>
<tr>
<td>Sample 3</td>
<td>8843</td>
<td>93.5</td>
</tr>
</tbody>
</table>

% Recovery - 85.9 109.5 106.2 129.6 125.4 118.0

10.3. Imprecision

<table>
<thead>
<tr>
<th>Mean value (ng/ml)</th>
<th>Reproducibility (%)</th>
<th>Within assay variation (%CV)</th>
<th>Between assay variation (%CV)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>8.4</td>
<td>9.9</td>
<td>13.2</td>
</tr>
<tr>
<td>Sample 2</td>
<td>5.9</td>
<td>6.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.9</td>
<td>2.4</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* The between assay variation is computed from sample data series made of concentrations determined using either the classical or the sensitive method.

10.4. Recovery test

When sera of known Rat TSH contents have their Rat TSH supplemented by addition of purified protein, a satisfactory correlation between added and assayed protein must be evidenced.

<table>
<thead>
<tr>
<th>Added Rat TSH (ng/ml)</th>
<th>Assayed Rat TSH (ng/ml)</th>
<th>Rat TSH recovered (ng/ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>1.83</td>
<td>2.38</td>
<td>3.45</td>
</tr>
<tr>
<td>Expected Rat TSH (ng/ml)</td>
<td>0.55</td>
<td>1.62</td>
<td>2.49</td>
</tr>
</tbody>
</table>

10.5. Linearity: Dilution test

The dilution test (dilution with CAL 0) indicates that there is immunological identity between the Rat GH present in serum and the Rat GH used to calibrate the standard curve.

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>Expected Rat TSH (ng/ml)</th>
<th>Assayed Rat TSH (ng/ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>6.05</td>
<td>3.62</td>
</tr>
<tr>
<td>2</td>
<td>4.03</td>
<td>3.03</td>
<td>2.02</td>
</tr>
<tr>
<td>3</td>
<td>1.01</td>
<td>0.76</td>
<td>0.5</td>
</tr>
</tbody>
</table>

11. Bibliography

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