Radioimmuno assay (RIA) for the quantitative determination of thyroxine-binding globulin (TBG) in human serum (Coated Tube System)

Article number: 53R.100 (100 determinations)

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Contents of the Kit

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity for 100 det.</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1 x 10.5 mL vial</td>
<td>Tracer (purified by HPLC), Iodine-125 thyroxine-binding globulin (TBG), red coloured, ready for use, activity: &lt; 180 kBq</td>
</tr>
<tr>
<td>C</td>
<td>2 x 50 coated tubes</td>
<td>Coated tubes, coated with anti-mouse antibody (polyclonal, goat), ready for use</td>
</tr>
<tr>
<td>L</td>
<td>1 x 22 mL vial</td>
<td>Anti-TBG antibody (monoclonal, mouse), ready for use</td>
</tr>
<tr>
<td>W</td>
<td>1 x 11 mL vial</td>
<td>B·R·A·H·M·S Washing solution universal, concentrate, 11 mL for 550 mL dist. water</td>
</tr>
<tr>
<td>S1 – S5</td>
<td>5 x 0.4 mL vials</td>
<td>TBG standards (human serum), ready for use, concentrations: 5; 10; 20; 40; 80 mg TBG/L</td>
</tr>
<tr>
<td>K1, K2</td>
<td>2 x 0.4 mL vials</td>
<td>Control sera 1 and 2 (human serum), ready for use, for further details see leaflet enclosed</td>
</tr>
</tbody>
</table>

Instruction Manual B·R·A·H·M·S TBG RIA

Date: 2012-01-16

(This version supersedes all earlier instruction manuals.)

Content changes versus previous version

- Insert the address of ALPCO

B·R·A·H·M·S Service

Address

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Switchboard

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E-Mail: info@brahms.de

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Thermo Fisher Scientific B·R·A·H·M·S LLC
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Middletown, Virginia 22645
Phone: 800.232.3342
Fax: 540.869.8126
e-Mail: techservice.mgc@thermofisher.com

Internet

www.thermoscientific.com/brahms
www.thermoscientific.com/procalcitonin
www.thermoscientific.com/kryptor

Radioactive

Total: < 180 kBq (REF 53R.100)

For research use only. Not for use in diagnostic procedures.
Introduction

BR·A·H·M·S TBG RIA is a competitive radioimmuno assay (RIA) for the quantitative determination of thyroxine-binding globulin (TBG) in human serum using coated tube technique.

This assay system is based on the rapid competition of unlabelled antigen of serum samples or standards and radiolabelled antigen (tracer) for the binding sites of the antigen-specific antibody (1. antibody), so during incubation antigen-antibody complexes are formed.

The concentration of the tracer (relative excess as compared to the specific antibody) and the concentrations of the antigen-specific antibody are constant in all tubes within the assay. Consequently, the only variable parameter of the system is the concentration of unlabelled antigen (standards or patient sera). As the concentration of unlabelled antigen in the sample increases, the binding of the competing tracer molecules to the antigen-specific antibody is inhibited, i.e., the portion of the radiolabelled antigen (tracer) is reversely proportional to the concentration of unlabelled antigen in the sample. During incubation a representative part of the antigen-antibody complexes are bound by an inner surface tube fixed antibody – which is directed against the antigen-specific antibody – and therefore separated from non-binding compounds.

After the incubation period non-bound compounds are removed by decanting the liquid phase. Remaining radioactivity in the tube is measured after washing. Subsequently a radioactivity-concentration-profile (standard curve) is constructed by means of the known concentrations of unlabelled antigen (standards) and the respective radioactive signal. Radioactivity values of patient sera correspond with their antigen concentration.

Important Notes

This kit contains materials of human origin (e.g. human serum). These materials have been screened for HBsAg, HIV I/II antibodies, and HCV antibodies; all tests were negative. However, the reagents and patient samples should be handled with care, as all materials of human origin are potentially hazardous.

The product contains the nuclide 125-iodine as an open radioactive substance. Ionising radiation in the form of photon radiation is emitted at an energy of approx. 30 keV. The half-life is 60 days. The product must be stored protected, and safeguarded against loss. It must be disposed of as radioactive waste.

To guarantee adequate radiation protection, the uptake of radioactive substances into the body should be avoided. Work rooms should be ventilated. Eating, drinking and smoking are prohibited. When working, an apron and protective gloves must be worn. Avoid remaining unnecessarily in the vicinity of radiation sources. It is recommended that the personal dose is calculated using appropriate measuring facilities such as film dosimeters.

The following kit reagents contain the preservative sodium azide at concentrations < 0.1 per cent by weight: tracer, antibody, standards, and control sera. These reagents should not be swallowed or allowed to come in contact with the skin or mucous membranes.

Our Customer Service Department, phone: +49 (0)3302/883 300, will gladly send the reagent-specific EU Safety Data Sheets in accordance to regulation 1907/2006-EC upon request.

In the event that glass vials are included in the reagent kit, we explicitly point out that there will be a breakage hazard, and consequently a risk of injury.

The reagents must be disposed of according to the specifications of local authorities.

Quality control: National quality assurance guidelines for quantitative tests in the medical laboratory (current version) must be complied with. For instance, test accuracy and precision can be monitored by means of laboratory in-house and/or commercially available control materials. If unacceptable control values are obtained, proceed as outlined in standard laboratory diagnostic procedures to determine the cause and implement corrective measures.

Bibliography

Test Procedure

Incubation Scheme

1. Number  test tubes (a, b)  T  1 – 5  6 etc.
2. Pipette  standards µL – 20 – 
   serum samples µL – – 20 
3. Pipette  tracer µL 100 100 100 
4. Pipette  antibody µL – 200 200 
5. Incubate  2 hours at room temperature (17...27°C) on orbital shaker (170 – 250 rpm) 
6. Aspirate or decant 
7. Pipette  washing solution mL – 2 2 
8. Aspirate or decant 
9. Measure radioactivity (Recommended counting time: 1 min)

Calculation of results

Specimen Handling

It is recommended to use serum. If plasma is used, separate reference values should be created.

Bilirubin and hemolysis are of no influence on the result of the test. Lipemic sera should not be used, because lipemia displays a trend to lower values.

If samples cannot be analyzed immediately, they can be stored for 3 days at 2...8°C or for longer periods of time at – 20 °C. Repeated freezing and thawing must be avoided.

Notes on Test Execution

Do not use any reagents that have exceeded the expiration date printed on the label.

The individual components of the kit are perfectly attuned to each other. If components from different batches are exchanged or mixed, B·R·A·H·M·S GmbH can assume no liability for the accuracy of results.

In large test series, reagents of the same batch designation are pooled.

The indicated sequence of steps must be followed.

Patient samples with a concentration above the measuring range are to be rated as “> highest standard”. The result must not be extrapolated. The patient sample in question should be diluted and retested. Further information can be obtained from B·R·A·H·M·S GmbH customer service.

Stability and Storage Conditions

Store all reagents and the coated tubes at 2 to 8 °C in their original shipping containers until directly prior to use. Observe the expiry dates specified on the main container and the vial labels. The shelf life of the kit is determined by the shelf life of the tracer.

Diluted washing solution may be used for up to 4 weeks if stored at 2...8°C. Contaminated washing solution must not be used. This is the case either if the liquid is clouded or the pH value is < 6.
Calculation of Results
For computer-aided evaluation, an evaluation programme (spline/unsmoothed) must be selected that suits the specific combination of processor and measuring equipment used.
When calculating the results without assistance of a computer, it is recommended to plot the mean count rates of the standards (ordinate, logarithmic) against the corresponding TBG concentrations (mg TBG/L, abscissa, logarithmic), in order to obtain a standard curve. The mean count rates of the unknown samples are then used to determine their corresponding TBG concentrations directly in mg TBG/L.
For technical support please contact B·R·A·H·M·S GmbH customer service or your local distributor.
For technical support, please contact the customer service of B·R·A·H·M·S GmbH or the appropriate distribution partner / sales representative.

Example

<table>
<thead>
<tr>
<th>Test tube</th>
<th>cpm (a)</th>
<th>cpm (b)</th>
<th>cpm (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total activity T</td>
<td>71 905</td>
<td>69 734</td>
<td>70 819</td>
</tr>
<tr>
<td>Standard 1 (5 mg/L)</td>
<td>11 966</td>
<td>11 996</td>
<td>11 981</td>
</tr>
<tr>
<td>Standard 2 (10 mg/L)</td>
<td>6 733</td>
<td>6 926</td>
<td>6 830</td>
</tr>
<tr>
<td>Standard 3 (20 mg/L)</td>
<td>3 547</td>
<td>3 584</td>
<td>3 565</td>
</tr>
<tr>
<td>Standard 4 (40 mg/L)</td>
<td>1 910</td>
<td>1 960</td>
<td>1 936</td>
</tr>
<tr>
<td>Standard 5 (80 mg/L)</td>
<td>1 020</td>
<td>1 077</td>
<td>1 049</td>
</tr>
<tr>
<td>Serum sample 6</td>
<td>4 103</td>
<td>4 287</td>
<td>4 195</td>
</tr>
</tbody>
</table>

· Interferences

<table>
<thead>
<tr>
<th>Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>no significant effect up to 9.6 g/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>no significant effect up to 20 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>no significant effect up to 600 mg/dL</td>
</tr>
</tbody>
</table>
## Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Usage</th>
<th>Symbol</th>
<th>Usage</th>
<th>Symbol</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Manufacturer" /></td>
<td>Manufacturer</td>
<td><img src="image2" alt="CE" /></td>
<td>CE Conformity Marking According to Directive 98/79/EC on In Vitro Diagnostic Medical Devices</td>
<td><img src="image3" alt="50" /></td>
<td>Contains sufficient for (Number of) tests, e.g. 50</td>
</tr>
<tr>
<td><img src="image4" alt="Use by" /></td>
<td>Use by</td>
<td><img src="image5" alt="Temperature Limitation" /></td>
<td>Temperature Limitation</td>
<td><img src="image6" alt="REF" /></td>
<td>Article Number/ Catalogue Number</td>
</tr>
<tr>
<td><img src="image7" alt="Green Dot" /></td>
<td>Green Dot according to German Law</td>
<td><img src="image8" alt="CAL" /></td>
<td>Standard</td>
<td><img src="image9" alt="CAL SET" /></td>
<td>Standard set</td>
</tr>
<tr>
<td><img src="image10" alt="Control Serum" /></td>
<td>Control Serum</td>
<td><img src="image11" alt="Ab" /></td>
<td>Antiserum, antibody</td>
<td><img src="image12" alt="SOLID PHASE 50x" /></td>
<td>Solid phase; 50 Coated tubes</td>
</tr>
<tr>
<td><img src="image13" alt="Batch code" /></td>
<td>Batch code</td>
<td><img src="image14" alt="Tracer / Enzyme conjugate" /></td>
<td>Tracer / Enzyme conjugate</td>
<td><img src="image15" alt="Radioactive" /></td>
<td>Radioactive</td>
</tr>
</tbody>
</table>

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Instruction manual (Version R06us-ruo)

Page 5 of 5