Immunoradiometric assay (IRMA) for the quantitative determination of thyroglobulin (h-Tg) in human serum (Coated Tube System)

Article number: 110.1 (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>2 x 10.5 mL vials</td>
<td>Tracer, 125I-labelled anti-h-Tg antibody (monoclonal, mouse), red colored, ready for use, activity: &lt; 404 kBq per vial</td>
</tr>
<tr>
<td>C</td>
<td>2 x 50 tubes</td>
<td>Coated tubes, coated with anti-h-Tg antibody (polyclonal, rabbit), ready for use</td>
</tr>
<tr>
<td>D</td>
<td>1 x 15 mL vial</td>
<td>Sample incubation buffer</td>
</tr>
<tr>
<td>G</td>
<td>1 x 1.8 mL vial</td>
<td>Tg-free serum, ready for use</td>
</tr>
<tr>
<td>W</td>
<td>1 x 40 mL vial</td>
<td>B·R·A·H·M·S Washing solution universal, concentrate, 40 mL (dilute 40 mL concentrate with 2 l distilled water)</td>
</tr>
<tr>
<td>S1 – S6</td>
<td>6 x 0.5 mL vials</td>
<td>Tg standards (serum), ready for use</td>
</tr>
<tr>
<td>K1, K2</td>
<td>2 x 0.5 mL vials</td>
<td>Control sera 1 and 2 (human serum), ready for use, see enclosed leaflet for concentrations</td>
</tr>
<tr>
<td>R50</td>
<td>1 x 10 mL vial</td>
<td>Buffer for Tg recovery (buffer-serum mixture), ready for use, blue colored, see enclosed leaflet for concentration</td>
</tr>
<tr>
<td>R1</td>
<td>1 x 10 mL vial</td>
<td>Separately available with article number 11048: Buffer for Tg recovery (buffer-serum mixture), ready for use, yellow colored</td>
</tr>
</tbody>
</table>

Instruction Manual B·R·A·H·M·S Tg-pluS RIA

Date: 2012-01-16
(This version supersedes all earlier instruction manuals.)

Content changes versus previous version

- Insert the address of ALPCO

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www.thermoscientific.com/kryptor

B·R·A·H·M·S GmbH
B·R·A·H·M·S Tg-pluS RIA
Instruction manual (Version R07us)
Introduction

Intended use(s):
B·R·A·H·M·S Tg-pluS RIA is a immunoradiometric assay (IRMA) for the quantitative determination of thyroglobulin in human serum. It is intended to aid in the monitoring for the presence of local or metastatic thyroid tissue in patients who have had thyroid gland ablation (by surgery with or without radioiodine therapy).

B·R·A·H·M·S Tg-pluS RIA is also indicated for detecting the presence of thyroid tissue in scans after recombinant thyrotropin (TSH) stimulation or thyroid hormone withdrawal.

B·R·A·H·M·S Tg-pluS RIA includes a recovery test to aid in the detection of interfering anti-thyroglobulin antibodies or the substances.

Clinical:
The physiological function of Tg, a globular iodine-containing glycoprotein that occurs in the thyroid gland, is to provide the substrate for synthesis of the thyroid hormones tetraiodothyronine (T₄) and triiodothyronine (T₃). As in other glycoproteins, Tg predictably demonstrates carbohydrate-dependent microheterogeneity, the variability of which is dependent on the degree of iodination of the molecule and differences in differentiation between normal and malignant thyroid tissue.

Tg is synthesized only in normal and malignant differentiated thyroid tissue, provided the latter can be classified as such. Thus, the synthesis, storage and secretion of Tg is organ-specific, i.e., tissue-specific.

In healthy individuals without thyroid disorders, small amounts of Tg (2 to 70 ng/mL) are constantly excreted into the circulated blood and can be detected in serum using a sensitive immunoassay. Most non-malignant functional disorders of the thyroid lead to an increase in serum Tg levels. However, this does not make it possible to diagnose the type of disease, because the extent of increase in the Tg level does not correlate with the type of pathological changes in the thyroid. Elevated serum Tg levels have been found to occur in various thyroid diseases such as Graves’ disease, Hashimoto’s disease, nontoxic diffuse goiter, etc. In addition to diseases that disturb the morphological integrity of the thyroid gland, various other conditions can also affect the serum Tg level, e.g., the menstrual cycle, pregnancy, smoking, iodine deficiency, growth hormones, certain medications, etc. Since the Tg gene is regulated by TSH (cAMP acts as the second messenger agonist), TSH has a stimulatory effect on the Tg level, whereas the administration of thyroid hormone usually reduces Tg levels in serum.

Clinical use

Due to its tissue specificity, i.e., organ specificity, the main clinical application of serum Tg determination is postoperative monitoring of patients with differentiated thyroid carcinoma (papillary, follicular, or oncocytic). Serum Tg assays are a valuable tool for early detection or exclusion of metastases and tumor recurrence, for postoperative monitoring after thyroidectomy, and for determining the effectiveness of subsequent radioiodine therapy.

After a complete thyroidectomy and radioiodine removal - with patients free of metastases and recurrences (complete remission) – the serum Tg is usually < 1 ng Tg/mL or can no longer be detected even with TSH stimulation.

Tg determination is further clinically indicated for clarification of a tentative diagnosis of factitious hyperthyroidism and for differential diagnosis of neonatal hypothyroidism.

Principle of measurement using B·R·A·H·M·S Tg-pluS RIA

B·R·A·H·M·S Tg-pluS RIA is an immunoradiometric two-step assay for determination of the amount of thyroglobulin (Tg) in human serum. Two antigen-specific antibodies that recognize different binding sites on the antigen (Tg) are used in excess.

In the first stage of incubation the antigen binds to the antibodies (polyclonal, rabbit) fixed to the inside of the tubes. By washing the serum twice all serum components and surplus antigens are washed out. In the second stage of incubation the radioactive tracer (marked antibody, monoclonal, mouse) reacts with the bound antigen, forming a sandwich complex which clings to the side of the tube. By washing the serum three times the remaining surplus tracer is removed. Following this, the radioactivity of the tubes is measured. Radioactive counts are directly proportional to the Tg concentration of the relevant sample.

Using the enclosed standards (known concentrations of antigen) a radioactivity concentration profile (standard curve) can now be established. The measured radioactivity of the patient samples is used to determine their relevant Tg concentration.

Recovery test

Anti-Tg antibodies or unspecific effects in a patient’s serum can interfere with serum thyroglobulin assays. Consequently, one should test the sera for such interferences by carrying out a recovery test as follows.

In parallel to the original sample 200 µL of Recovery Buffer R50 are added to 100µL of the serum to be tested and determined in the same assay run. The recovery reference (D₅₀) is determined using Tg-free serum (G) and R50. With unimpaired recovery (100%), i.e. if no factors are present in the patient serum that interfere with Tg determination, this result in the B·R·A·H·M·S Tg-pluS RIA is approximately 50 ng/mL [100 ng/mL according to CRM 457] above the Tg level of the corresponding original sample. Taking into consideration any pipetting inaccuracies, recoveries between 70% and 130% are considered valid. Levels of < 70% or > 130% are due to interference and the Tg level of the relevant original sample is considered invalid. As a rule, a recovery of < 70% can be found with IRMA systems.

Recovery (in %) in the serum sample

\[ \frac{\text{ng Tg/mL (Pₐ)} - \text{ng Tg/mL (Pₐ)}}{\text{ng Tg/mL (Dₐ)}} \times 100 = \% \text{ recovery} \]

The concentration for Dₐ is included in the package insert and is approximately 50 ng Tg/mL [100 ng/mL according to CRM 457]. It must also be determined for every case. Details included in the package insert are only to be used as a guideline.

Recovery and postoperative monitoring of patients with differentiated thyroid carcinoma

Using the usual recovery (R₅₀; ≈ 50 ng/mL) relevant interfering effects may not be found in every case for samples with low Tg level caused by the large difference in Tg concentration between sample and recovery sample.

For these cases an optional recovery sample (must be ordered additionally, article number 11048) with low Tg concentration (R₁, ≈ 1 ng/mL) is available. This recovery sample additionally offers high reliability in postoperative monitoring of patients with differentiated thyroid carcinoma.

Use of recovery sample with low Tg concentration is recommended for patient sera with Tg values up to 5 ng/mL. The recovery sample R₁ will be used like the recovery sample R₅₀ included in the kit.

"High dose hook" effect

With B·R·A·H·M·S Tg-pluS RIA concentrations of up to 200,000 ng Tg/mL do not result in a “high dose hook” effect.
Standardisation and Reference Ranges

Standardisation of the B·R·A·H·M·S Tg-pluS RIA is based on the international standard Certified Reference Material (CRM) 457. 1 ng in the B·R·A·H·M·S Tg-pluS RIA is equivalent to 2 ng of CRM. This ensures consistency of Tg measurements with those of the previous product B·R·A·H·M·S Tg-S RIA.

Normal Tg values can be distinctly lower in areas with sufficient or high alimentary iodine supply. As a rule of thumb healthy persons have a serum level of approximately 1 ng/mL thyroglobulin per 1 mL of thyroid tissue.

In patient-free of metastases and recurrences (complete remission) after a complete thyroidectomy and radiiodine removal for differentiated carcinoma of the thyroid - the serum Tg is usually < 1 ng/mL or can no longer be detected even with TSH stimulation.

The post-operative growth of primary metastases after a complete thyroidectomy and radiiodine therapy is generally accompanied by postoperative measurable high serum Tg concentrations. Measurable and increasing concentrations of Tg are an early warning signal of recurrence or metastasis, particularly if the Tg level is already measurable with TSH suppressive therapy. Increasing Tg-values provide an important signal for recurring or still existing neoplasias.

It is recommended that each laboratory establish its own "normal ranges" based on representative patient collectives and/or test the validity of the manufacturer's commercial test kit data using samples from a relevant patient population. This makes it possible to identify regional features and variations in the prevalence of certain metabolic thyroid diseases and dysfunctions and also serves as a test of the methodological quality of the different laboratories and test kits. Therefore, the data given for the B·R·A·H·M·S Tg-S RIA should be treated as guideline values only.

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.

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 label. The shelf life of the kit is determined by the shelf life of the manufacturer's commercial test kit data using samples from a relevant patient population. This makes it possible to identify regional features and variations in the prevalence of certain metabolic thyroid diseases and dysfunctions and also serves as a test of the methodological quality of the different laboratories and test kits. Therefore, the data given for the B·R·A·H·M·S Tg-S RIA should be treated as guideline values only.

The following kit reagents contain the preservative sodium azide at concentrations of < 0.1 percent by weight: tracer, standards, recovery sample and control sera. These reagents should not be swallowed or allowed to come in contact with the skin or mucous membranes. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush drains with generous amounts of cold water to prevent azide buildup.

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.

The results, obtained from this assay should always be assessed in combination with the clinical examination, patients medical history, and other findings before momentous actions will be prefaced.

Stability and Storage Conditions

Store all reagents and 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.

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Diluted washing solution may be used for up to 4 weeks if stored at 2…8°C. Contaminated washing solution must not be used. Diluted washing solution that is clouded or has a pH value < 6 must not be used.

Bibliography

Assay Characteristics

· Intra-assay Precision
The patient values listed below have been determined in an assay-run on 10-fold determination using fresh tracer.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mean [ng/mL]</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.39</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>0.64</td>
<td>5.2</td>
</tr>
<tr>
<td>3</td>
<td>1.32</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>21.3</td>
<td>1.5</td>
</tr>
<tr>
<td>5</td>
<td>54.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

· Inter-assay Precision
The patient values listed below have been determined in 10 assay-runs in duplicate using fresh tracer.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mean [ng/mL]</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>9.9</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>1.75</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>21.0</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>55.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

· Sensitivity
The functional assay sensitivity (20 % inter-assay coefficient of variation) is 0.1 ng/mL. (values below 0.10 ng/ml should be declared as <0.10 ng/ml)

· Dilution

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dilution</th>
<th>Observed value [ng/mL]</th>
<th>Expected value [ng/mL]</th>
<th>Observed / expected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:2</td>
<td>56.40</td>
<td>26.80</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>28.20</td>
<td>13.70</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>7.05</td>
<td>6.99</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>3.53</td>
<td>3.47</td>
<td>98.3</td>
</tr>
<tr>
<td></td>
<td>1:32</td>
<td>1.76</td>
<td>1.72</td>
<td>97.7</td>
</tr>
<tr>
<td>2</td>
<td>1:2</td>
<td>88.60</td>
<td>41.60</td>
<td>93.9</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>44.30</td>
<td>21.30</td>
<td>96.2</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>11.08</td>
<td>11.00</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>1:16</td>
<td>5.54</td>
<td>5.44</td>
<td>98.2</td>
</tr>
<tr>
<td></td>
<td>1:32</td>
<td>2.77</td>
<td>2.76</td>
<td>99.6</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 6.2 g/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>no significant effect up to 200 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>no significant effect up to 300 mg/dL</td>
</tr>
</tbody>
</table>

· Method comparison
B·R·A·H·M·S Tg-pluS RIA was compared to a commercially available chemiluminescent thyroglobulin immunoassay in 133 patients with thyroid gland ablation without serum thyroglobulin autoantibodies. Concordance testing yielded the following results:

<table>
<thead>
<tr>
<th>Comparison Method</th>
<th>&lt;5.0 ng/mL</th>
<th>5.0 – 9.9 ng/mL</th>
<th>10.0 – 29.9 ng/mL</th>
<th>30.0 – 59.9 ng/mL</th>
<th>≥60.0 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>B·R·A·H·M·S Tg-pluS RIA</td>
<td>78</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&gt;2.5 ng/mL</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.0 – 14.9 ng/mL</td>
<td>0</td>
<td>1</td>
<td>19</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>&gt;15.0 – 29.9 ng/mL</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>≥30.0 ng/mL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>

Note: B·R·A·H·M·S Tg-pluS RIA is calibrated against CRM 457 at a ratio of 0.5:1 whereas the comparison method is calibrated against CRM 457 at a 1:1 ratio.

Agreement for results less than 30 ng/mL determined with B·R·A·H·M·S Tg-pluS RIA = 100%
Agreement for results greater than 30 ng/mL determined with B·R·A·H·M·S Tg-pluS RIA = 93.3%

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 with or without assistance of a computer, plot the mean count rates of the standards (ordinate, logarithmic) against the corresponding Tg concentrations (abscissa, logarithmic), in order to obtain a standard curve. The mean count rates of the unknown samples are then used to determine their corresponding Tg concentrations.

B6/T is calculated to check the binding capacity of the IRMA system:

\[
\frac{B_6}{T} = \frac{\text{mean count rate } 6 \ a, b}{\text{mean count rate } T \ a, b} \times 100
\]

For the assay conditions described above, the B6/T should range from 35 – 65 %.

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 tubes</th>
<th>cpm (a)</th>
<th>cpm (b)</th>
<th>cpm (mean)</th>
<th>B/T (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total activity T</td>
<td>369,447</td>
<td>368,160</td>
<td>368,804</td>
<td>0.08</td>
</tr>
<tr>
<td>Standard 1 (0.15 ng/mL)</td>
<td>285</td>
<td>280</td>
<td>283</td>
<td>0.08</td>
</tr>
<tr>
<td>Standard 2 (0.8 ng/mL)</td>
<td>952</td>
<td>930</td>
<td>941</td>
<td>0.26</td>
</tr>
<tr>
<td>Standard 3 (4.0 ng/mL)</td>
<td>4,270</td>
<td>3,978</td>
<td>4,124</td>
<td>1.12</td>
</tr>
<tr>
<td>Standard 4 (20 ng/mL)</td>
<td>21,504</td>
<td>20,469</td>
<td>20,987</td>
<td>5.69</td>
</tr>
<tr>
<td>Standard 5 (100 ng/mL)</td>
<td>105,587</td>
<td>104,136</td>
<td>104,862</td>
<td>28.4</td>
</tr>
<tr>
<td>Standard 6 (250 ng/mL)</td>
<td>202,170</td>
<td>198,997</td>
<td>200,584</td>
<td>54.4</td>
</tr>
<tr>
<td>Recovery sample D_{R50}</td>
<td>56,635</td>
<td>58,102</td>
<td>57,368</td>
<td>52</td>
</tr>
</tbody>
</table>

### Standard Curve

- **X-axis:** Tg (ng/ml)
- **Y-axis:** CPM

### Test Procedure

#### Incubation Scheme

1. **Number coated tubes (a, b)**
   - T: 1 – 6, I, II, D_{R50}, P_1, ..., P_n, P_{last}...
2. **Pipette Standards S1 – S6**
   - µL: 100
   - Tg-free serum G µL: 100
   - Patient sample 1 ... n µL: 100
3. **Pipette Buffer D (PIP)**
   - µL: 200
   - Recovery samples R50 * µL: 200
4. **Incubate**
   - Agitate briefly using a sample mixer to ensure good blending.
   - **Incubate 18 ± 4 h without shaking at room temperature (17 – 27 ºC).**
5. **Pipette Washing solution mL**
   - 2
6. **Decant**
   - Decant the liquid completely and turn the tubes upside down on blotting paper.
   - **Repeat steps 5. and 6. once.**
7. **Pipette Tracer A**
   - µL: 200
   - Incubate 2 h ± 30 min with shaking (170 – 300 rpm) at room temperature (17 – 27 ºC).
8. **Pipette Washing solution mL**
   - 2
9. **Decant**
   - Decant the liquid completely and turn the tubes upside down on blotting paper.
   - **Repeat steps 9. and 10. two times, turn the tubes upside down on blotting paper for minimum 10 min.**
10. **Measure radioactivity**
    - Recommended counting time: 1 minute

#### Calculation of results

*optional R1*

### Specimen Handling

Store samples for 3 days at 2…8°C or for longer periods of time at – 20°C if samples cannot be analyzed immediately. 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.
Test Procedure

1. Preparations
   - Allow all kit components and patient samples to warm up to room temperature.
   - Agitate all liquid reagents, including patient sera, gently before use (avoid foam formation).
   - Prepare washing solution: dilute 40 mL concentrate with distilled water to yield 2 L.
   - Label the coated tubes for standards, controls, recovery samples and serum samples in a useful way which avoid any mix-up. Use two uncoated tubes for measuring total activity (e.g. \(T_a, T_b\)).

Note: If relatively high Tg values (> 250 ng Tg/mL) are expected for any patient's serum, the original sample should be diluted before starting the assay. The dilution should be done using the Tg-free serum; do not use any buffers for this purpose. The recovery test is then carried out as described using the diluted sample.

2. The following stages of pipetting are carried out:
   - 100 µL standards with an increasing concentration of Tg into the standard tubes.
   - 100 µL controls into the control tubes.
   - 100 µL Tg free serum (G) into the recovery reference tubes.
   - 100 µL serum sample into the sample tubes and into the recovery tubes.

3. Followed by the pipetting of:
   - 200 µL sample incubation buffer (D) into standard tubes, control tubes and sample tubes.
   - 200 µL buffer with recovery sample R50 (optional R1) into the recovery reference tubes and into the recovery tubes.

4. Then the test tubes are briefly agitated using a sample mixer to ensure good blending. Cover the test tubes with adhesive film and incubate at room temperature overnight (18 ± 4 hours) without agitation.

5. After incubation 2 mL of the ready to use washing solution is added to each test tube.

6. Decant the test tubes and place upside down on adsorbent paper so that remaining liquid can drain.

Steps 5 and 6 are repeated one time and the tubes are left to stand upside down for a minimum of 10 minutes after decanting.

7. 200 µL tracer (A) is pipetted into each tube (including the tubes used for total activity). Tubes used for total activity are removed from further processing until measurement of radioactivity is done.

8. Cover the test tubes with adhesive film and incubate for 2 hours ± 30 minutes at room temperature (17...27°C) with shaking (170 – 300 rpm).

9. 2 mL of the ready to use washing solution is added to each test tube – excluding tubes used for total activity – after incubation.

10. All tubes – excluding tubes used for total activity – are decanted and placed upside down on adsorbent paper to drain.

Steps 9 and 10 are repeated twice and the tubes are left to stand upside down on adsorbent paper for a minimum of 10 minutes after decanting for the last time.

11. Radioactivity of each tube – including tubes used for total activity – is measured in a gamma counter. Recommended measuring time: 1 minute.

Carefully follow the manufacturer's instructions. Improper handling of the reagents may render the test results invalid. B·R·A·H·M·S GmbH is not liable for faulty test results arising from improper storage, use or handling.

Additionally required
   - micropipettes (100 µL, 200 µL)
   - sample mixer (e.g. Vortex)
   - gamma counter
   - dispenser (e.g. 10 mL) for B·R·A·H·M·S Washing solution universal, concentrate, 40mL (dilute 40 mL concentrate with 2 L distilled water)
<table>
<thead>
<tr>
<th>Symbol</th>
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