Serotonin (Research) RIA

For the quantitative determination of serotonin in various biological sample types and volumes

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 17-SERHU-R50-RES
Size: 100 determinations

This Protocol is for Reference Purposes Only. DO NOT use this copy to run your assay; use the protocol included with the kit ONLY.
1. Principle of the test
Serotonin is acylated quantitatively. The subsequent assay procedure follows the basic principle of radioimmunoassays, involving competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of $^{125}$I-labeled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. After the system is in equilibrium, the antibody bound radioactivity is precipitated with a second antibody in the presence of polyethylene glycol. The precipitate is counted in a gamma counter. Quantification of unknown samples is achieved by comparing their activity with a reference curve prepared with known standards.

2. Storage and stability
The reagents should be stored at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels.

3. Contents of the kit

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Quantity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA 0901</td>
<td>Standard A</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 0902</td>
<td>Standard B</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 0903</td>
<td>Standard C</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 0904</td>
<td>Standard D</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 0905</td>
<td>Standard E</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 0906</td>
<td>Standard F</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 0910</td>
<td>Serotonin Antiserum</td>
<td>1 x 5.25 mL</td>
<td>from rabbit, ready for use, blue colored, blue screw cap</td>
</tr>
<tr>
<td>BA 0911</td>
<td>Acylation Buffer</td>
<td>1 x 30 mL</td>
<td>ready for use</td>
</tr>
<tr>
<td>BA 0920</td>
<td>$^{125}$I – Serotonin</td>
<td>1 x 5.5 mL</td>
<td>activity &lt; 200 kBq, ready for use, red colored, red screw cap</td>
</tr>
<tr>
<td>BA 0951</td>
<td>Control 1</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 0952</td>
<td>Control 2</td>
<td>1 x 4 mL</td>
<td>concentrated*</td>
</tr>
<tr>
<td>BA 3030</td>
<td>Precipitating Reagent</td>
<td>1 x 55 mL</td>
<td>ready for use, goat anti-rabbit serum in PEG phosphate buffer. Mix thoroughly before use!</td>
</tr>
<tr>
<td>BA 5937</td>
<td>Ascorbic Acid</td>
<td>1 x 2 mL</td>
<td>10% (w/v); ready for use</td>
</tr>
<tr>
<td>BA 5934</td>
<td>Acylation Plate</td>
<td>1 x 96 wells</td>
<td>ready for use, pre-coated with Acylation Reagent</td>
</tr>
</tbody>
</table>

* Dilute standards and controls 1+150 with the buffer/solvent, which is used for the experiment, containing 0.1 % (w/v) ascorbic acid.
Actually the following buffers/solvents are evaluated for use: Ringer buffer, PBS and 0.9% NaCl. Other buffers/solvents are suitable but should be evaluated before use!

4. Additional materials and equipment required but not provided in the kit
- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000µL)
- Plastic tubes (polypropylene, polystyrene) and suitable rack
- Orbital shaker (capable of shaking between 400-500 rpm)
- Centrifuge (preferable refrigerated) capable of at least 3,000 x g
- Suitable device for aspirating or decanting the tubes.
- Vortex mixer
- Gamma counter
- Distilled water

5. Sample collection and storage
Tissue homogenates, dialysates and other samples could be stored for 6 hours at 2 – 8 °C. For longer periods (up to 6 months) the samples should be stored at - 20°C.

⚠️ To protect Serotonin against oxidative degradation the samples should contain 0.1% ascorbic acid.

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6. **Test procedure**
Allow all reagents – with the exception of Precipitating Reagent – to reach room temperature and mix thoroughly by gentle inversion before use. Number the assay tubes (polystyrene or polypropylene) accordingly. Duplicate determinations are recommended.

⚠️ The use of glass tubes is not recommended for this assay.

6.1 **Preparation of reagents**

**Standards and controls**
The standards and controls have to be diluted 1+ 150 with buffer* (for example: 10 µL standard + 1.5 mL buffer).

⚠️ The standards have to be prepared freshly prior to the assay.

⚠️ * The buffer used for the respective experiment, enriched with 0.1 % (w/v) ascorbic acid.
Evaluated for Ringer buffer, PBS and 0.9% NaCl. Other buffers can be evaluated quite easily.

6.3 **Sample preparation and acylation**
1-20 µL of sample can be used with this assay. If less then 20 µl of sample is used, the volume has to be adjusted to a total volume of 20 µl with the corresponding buffer containing 0.1% (w/v) ascorbic acid.

1. Pipette 20 µL of the diluted standards, diluted controls, and 20 µL of the samples (diluted or undiluted) into the respective wells of the Acylation Plate.
2. Add 25 µL of Acylation Buffer to all wells.
3. Incubate for 30 minutes at RT (20-25°C) on a shaker (approx. 600 rpm).

⚠️ 40 µL of the acylated standards, controls and samples are needed for the subsequent RIA.

6.4 **Serotonin RIA**

1. Pipette 40 µL of the buffer used for the respective experiment into the tubes for the NSB.
2. Pipette 40 µL of acylated standards, controls and samples into the respective tubes.
3. Pipette 20 µL of the ¹²⁵I Serotonin into all tubes.
4. Pipette 20 µL of the Serotonin Antiserum into all tubes (except totals and NSB); mix thoroughly.
5. Cover tubes. Incubate for 15 - 20 hours (overnight) at 2 - 8 °C.
6. Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 500 µL into all tubes (except totals), and mix on a vortex.
7. Incubate for 15 minutes at 2 - 8 °C.
8. Centrifuge for 15 minutes at 3,000 x g, if possible in a refrigerated centrifuge.
9. Decant or aspirate the supernatant carefully (except totals). Blot the tubes dry and leave them upside for 2 minutes.
10. Count all tubes for 1 minute in a gamma counter.

7. **Calculation of results**

<table>
<thead>
<tr>
<th></th>
<th>Concentration of the diluted standards</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard</strong></td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>Serotonin (ng/mL)</td>
<td>0</td>
</tr>
<tr>
<td>Serotonin (nmol/L)</td>
<td>0</td>
</tr>
<tr>
<td>Serotonin (pg/sample volume)</td>
<td>0</td>
</tr>
<tr>
<td>Serotonin (pmol/sample volume)</td>
<td>0</td>
</tr>
</tbody>
</table>

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Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.

The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/(B0-NSB) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).

Use a non-linear regression for curve fitting (e.g. spline, 4-parameter, akima).

⚠️ The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor:

\[
\text{Correction factor} = \frac{20 \, \mu\text{L (volume of standards)}}{\mu\text{L (sample volume)}}
\]

**Example:** 10 µL of the sample are extracted and the concentration taken from the standard curve is 0.1 ng/mL serotonin.

Correction factor = \(\frac{20}{10} = 2\)

Final concentration of the sample = 0.1 ng/mL x 2 = 0.2 ng/mL serotonin

7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Controls with both normal and pathological levels should be used. The kit or other commercially available controls should be found within the established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

8. Assay characteristics

8.1 Sensitivity

<table>
<thead>
<tr>
<th>Serotonin</th>
<th>Sensitivity</th>
<th>0.05 ng/mL x C*</th>
</tr>
</thead>
</table>

\[C^* = \text{Correction factor} \text{ (refer to 7.)} \]

8.2 Specificity

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td>100</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>3.000</td>
</tr>
<tr>
<td>Melatonin</td>
<td>0.056</td>
</tr>
<tr>
<td>5-Hydroxyindole acetic acid</td>
<td>0.002</td>
</tr>
<tr>
<td>5-Hydroxy-2-carboxylic acid</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histidine</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tyramine</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5-Hydroxytryptophan</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

9. Advice on handling the test

9.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

It is recommended that each laboratory establishes its own reference intervals. This kit is for research use only. It is not for use in diagnostic procedures.
9.2 Complaints
In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a complaint form and return it completely filled in to the manufacturer.

9.3 Warranty
This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

9.4 Disposal
Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federal and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate material safety data sheets of the individual products are available upon request. The material safety data sheets correspond to the standard: ISO 11014-1.

9.5 Interference
Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

9.6 Precautions
Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
This kit contains $^{125}$Iodine (half life: 60 days), emitting ionizing $\alpha$ - (28 kev) and $\beta$- (35.5 kev) radiations. The radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. In no case the product must be administered to humans or animals.
All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radio safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.