Serotonin ELISA

For the quantitative determination of serotonin in platelets, serum and urine.

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

Catalog Number: 17-SERHU-E01-FST
Size: 96 Wells
Version: 2017-06-26 15.0-r - ALPCO November 17, 2017
Serotonin ELISA Fast Track

1. **Introduction**

1.1 **Intended use and principle of the test**

Enzyme immunoassay for the quantitative determination of serotonin in serum, urine and platelets.

In the first step, serotonin is quantitatively acylated. The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

1.2 **Background**

Serotonin (5-hydroxytryptamine) is an intermediate product of tryptophan metabolism and is located primarily in the enterochromaffin cells of the intestine (EC-cells), serotonergic neurons of the brain, platelets of the blood and is well established as a neurotransmitter in the central nervous system. EC-cell production accounts for 80% of the body's serotonin content. Serotonin is predominately metabolized to 5-hydroxyindoleacetic acid (5-HIAA), which is excreted by the kidneys.

2. **Procedural cautions, guidelines, warnings and limitations**

2.1 **Procedural cautions, guidelines and warnings**

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

(2) The principles of Good Laboratory Practice (GLP) have to be followed.

(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.

(4) All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.

(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.

(6) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.

(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.

(8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared and ready at the appropriate time.

(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.

(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.

(11) A calibrator curve must be established for each run.

(12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.

(13) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(14) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(15) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(16) For information on hazardous substances included in the kit please refer to Safety Data Sheets (SDS). The Safety Data Sheet for this product is made available directly on the ALPCO website or upon request.
(17) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations
Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances
Serum/Plasma
Samples containing precipitates or fibrin strands or which are hemolyzed or lipemic might cause inaccurate results.
24-hour urine
Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for urine samples.

2.2.2 Drug interferences
Please refer to “Sample collection and storage”.

2.2.3 High-Dose-Hook effect
No hook effect was observed in this test.

3. Storage and stability
Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials
4.1 Contents of the kit

**BA D-0023**
**REACT-TUBES**  Reaction Tubes* - Ready to use
Contents: Reaction Tubes in a resealable pouch
Volume: 2 x 50 tubes
*Instead of the Reaction Tubes, it is also possible to use 48 wells macrotiter plates for the sample preparation and acylation (please refer to 6.2). These plates (BA D-0033) are available upon request.

**BA E-0030**
**WASH-CONC50X**  Wash Buffer Concentrate - Concentrated 50x
Contents: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 mL/vial, light purple cap

**BA E-0045**
**CONJUGATE**  Enzyme Conjugate - Ready to use
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 mL/vial, red cap

**BA E-0055**
**SUBSTRATE**  Substrate - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 mL/vial, black cap

BA E-0080 STOP-SOLN Stop Solution - Ready to use
Contents: 0.25 M sulfuric acid
Volume: 1 x 12 mL/vial, light grey cap
Hazards Identification
H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

BA E-0931 SER-S-HIAA Serotonin Microtiter Strips - Ready to use
Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant

BA E-8910 SER-AS Serotonin Antiserum - Ready to use
Contents: Rabbit anti-serotonin antibody, blue colored
Volume: 1 x 12 mL/vial, blue cap

Standards and Controls - Ready to use

<table>
<thead>
<tr>
<th>Cat. no.</th>
<th>Component</th>
<th>Color/Cap</th>
<th>Concentration ng/mL</th>
<th>Concentration nmol/L</th>
<th>Volume/Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA R-8901</td>
<td>STANDARD A</td>
<td>white</td>
<td>0</td>
<td>0</td>
<td>4 mL</td>
</tr>
<tr>
<td>BA R-8902</td>
<td>STANDARD B</td>
<td>light yellow</td>
<td>15</td>
<td>85.1</td>
<td>4 mL</td>
</tr>
<tr>
<td>BA R-8903</td>
<td>STANDARD C</td>
<td>orange</td>
<td>50</td>
<td>284</td>
<td>4 mL</td>
</tr>
<tr>
<td>BA R-8904</td>
<td>STANDARD D</td>
<td>dark blue</td>
<td>150</td>
<td>851</td>
<td>4 mL</td>
</tr>
<tr>
<td>BA R-8905</td>
<td>STANDARD E</td>
<td>light grey</td>
<td>500</td>
<td>2840</td>
<td>4 mL</td>
</tr>
<tr>
<td>BA R-8906</td>
<td>STANDARD F</td>
<td>black</td>
<td>2500</td>
<td>14,175</td>
<td>4 mL</td>
</tr>
<tr>
<td>BA R-8951</td>
<td>CONTROL 1</td>
<td>light green</td>
<td></td>
<td></td>
<td>4 mL</td>
</tr>
<tr>
<td>BA R-8952</td>
<td>CONTROL 2</td>
<td>dark red</td>
<td></td>
<td></td>
<td>4 mL</td>
</tr>
</tbody>
</table>

Conversion: Serotonin (ng/mL) x 5.67 = Serotonin (nmol/L)
Contents: TRIS buffer with non-mercury preservatives, spiked with defined quantity of serotonin

BA E-8912 ACYL-REAG Acylation Reagent - Ready to use
Contents: Acylation reagent in dimethylsulfoxide
Volume: 1 x 3 mL/vial, green cap

BA E-8911 ACYL-BUFF Acylation Buffer - Ready to use
Contents: TRIS buffer with non-mercury preservative
Volume: 1 x 55 mL/vial, light grey cap

4.2 Additional materials and equipment required but not provided in the kit
- Calibrated precision pipettes to dispense volumes between 25 – 1000 µL
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer
- Timer
- Centrifuge capable of 200 xg, 4,500 xg, and 10,000 xg (for platelet samples)

⚠️ The assay can be performed with or without shaking. If a microtiter plate shaker is used, it should have the following characteristics: shaking amplitude 3 mm; approx. 600 rpm.

5. **Sample collection and storage**

**Repeated freezing and thawing of the samples should be avoided.**

Foods or liquids containing serotonin such as pineapple, eggplant, avocados, bananas, currants, kiwis, melon, mirabelles, plums, peaches, chocolate, gooseberries, tomatoes, or walnuts should be avoided 2 days before and including the day of the sample collection (24-hour urine). Selective Serotonin Reuptake Inhibitors (SSRIs) influence serotonin levels.

**Serum**

Collect blood by venipuncture (monovette or vacuette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Sample donors receiving anticoagulant therapy may require increased clotting time. Hemolyzed and lipemic samples should not be used for the assay.

Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 months) at -20 °C.

**Urine**

Spontaneous or 24-hour urine, collected in a bottle containing 10 – 15 mL of 6 M HCl, should be used. Determine the total volume of urine excreted during a period of 24 hours for calculation of the results. Storage: up to 24 hours at 2 – 8 °C, for longer periods (up to 6 months) at -20 °C. Avoid exposure to direct sunlight.

**Platelets**

More than 98 percent of the circulating serotonin is located in the platelets and is released during blood clotting. Blood must be collected by venipuncture in plastic tubes (monovette or vacuette) containing EDTA or Citrate as anticoagulant.

To obtain platelet-rich plasma (PRP) the samples are centrifuged for 10 minutes at room temperature (200 xg). Transfer the supernatant to another tube and count the platelets.

The platelet pellet is obtained by adding 800 µL of physiological saline to 200 µL of PRP (containing between 350,000 – 500,000 platelets/µL) and centrifugation (4,500 xg, 10 minutes at 4 °C). The supernatant is then discarded.

200 µL of water (deionized, distilled, or ultra-pure) is added to the pellet and mixed thoroughly on a vortex mixer. This suspension can be stored frozen for several weeks at < -20 °C.

After thawing of the frozen samples, centrifuge at 10,000 xg for 2 minutes at room temperature. 25 µL of the supernatant is used for the acylation reaction.
For the determination of Serotonin in platelet-poor plasma and cerebrospinal fluid the Serotonin Research™ ELISA (17-SERHU-E01-RES) should be used.

6. Test procedure for serum, urine and platelets

Allow all reagents and samples to reach room temperature. Measurement in duplicate is recommended. It is recommended to number the strips of the microwells before usage to avoid any mix-up.

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 mL.
Storage: 1 month at 2 – 8 °C

Acylation Reagent
The Acylation Reagent (BA E-8912) has a freezing point of 18.5 °C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used.

Serotonin Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Sample preparation and acylation of serum, urine and platelets

1. Pipette 25 µL of standards, controls and serum, urine or platelets into the respective Reaction Tubes.
2. Add 500 µL of Acylation Buffer to all tubes.
3. Add 25 µL of Acylation Reagent to all tubes.
4. Mix thoroughly and incubate for 15 min at RT (20 – 25 °C).

⚠ Take 25 µL of the prepared standards, controls and samples for the Serotonin ELISA

6.3 Serotonin ELISA
The use of a shaker is not mandatory. The alternative protocol without a shaker is highlighted in italics and shaded in grey.

1. Pipette 25 µL of the acylated standards, controls and samples into the appropriate wells of the Serotonin Microtiter Strips.
2. Pipette 100 µL of the Serotonin Antiserum into all wells.
3. Incubate 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).

Without use of a shaker: shake the Serotonin Microtiter Strips shortly by hand and incubate for 1 hour at RT (20 – 25 °C).
4. Discard or aspirate the contents of the wells. Wash the plate 3x by adding 300 µL of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.

5. Pipette 100 µL of the Conjugate into all wells.

6. Incubate for 15 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
   **Without use of a shaker:** incubate for 15 min at RT (20 – 25 °C).

7. Discard or aspirate the contents of the wells. Wash the plate 3x by adding 300 µL of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.

8. Pipette 100 µL of the Substrate into all wells.

9. Incubate for 15 ± 2 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
   **Without use of a shaker:** incubate for 15 min ± 2 min at RT (20 – 25 °C).
   **Avoid exposure to direct sunlight!**

10. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.

11. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm (if available a reference wavelength between 620 nm and 650 nm is recommended).

### 7. Calculation of results

<table>
<thead>
<tr>
<th>Measuring range</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.2 – 2500 ng/mL</td>
</tr>
</tbody>
</table>

The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of 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).

*This assay is a competitive assay. This means: the OD values are decreasing with increasing concentrations of the analyte. OD values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The concentrations for urine and serum samples can be read directly from the calibration curve.

**Calculation of serotonin in platelets**

The content of serotonin in platelets is normalized to the concentration per 10⁹ platelets.

*Illustrative example:*

Measured Serotonin concentration: 100 ng/mL

Number of the platelets in the PRP: 300,000 / µL = 0.3 x 10⁹ platelets/mL with serotonin content of 100 ng.

The resulting serotonin content in the platelets is:

333 ng/ 10⁹ platelets (100 ng serotonin x 1.0 x 10⁹ /0.3 x 10⁹)

**Conversion**

Serotonin (ng/mL) x 5.67 = Serotonin (nmol/L)

### 7.1 Quality control

The confidence limits of the kit controls are printed on the QC-Report.

### 7.2 Typical standard curve:
8. **Assay characteristics**

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Limit of Detection (LOD)</th>
<th>6.2 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Limit of Quantitation (LOQ)</td>
<td>10.2 ng/mL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Analytical Specificity (Cross-Reactivity)</th>
<th>Substance</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tryptamine</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Melatonin</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>5-Hydroxyindole acetic acid</td>
<td>&lt; 0.014</td>
</tr>
<tr>
<td></td>
<td>Phenylalanine</td>
<td>&lt; 0.014</td>
</tr>
<tr>
<td></td>
<td>Histidine</td>
<td>&lt; 0.019</td>
</tr>
<tr>
<td></td>
<td>Tyramine</td>
<td>&lt; 0.018</td>
</tr>
<tr>
<td></td>
<td>5-Hydroxytryptophan</td>
<td>&lt; 0.014</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precision</th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Range (ng/mL) mean ± SD</td>
<td>CV (%)</td>
</tr>
<tr>
<td>Serotonin Urine (n = 40)</td>
<td>1</td>
<td>140.7 ± 16.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>421.2 ± 38.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,560 ± 215.3</td>
</tr>
<tr>
<td>Serotonin Serum (n = 20)</td>
<td>1</td>
<td>101.3 ± 9.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>246.8 ± 31.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>667.5 ± 71.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Linearity</th>
<th>Range (ng/mL)</th>
<th>Serial dilution up to</th>
<th>Mean Linearity (%)</th>
<th>Range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin Urine</td>
<td>30 – 3,500</td>
<td>1:65</td>
<td>100</td>
<td>88 - 118</td>
</tr>
<tr>
<td>Serotonin Serum</td>
<td>40 – 3,000</td>
<td>1:33</td>
<td>96</td>
<td>80 -113</td>
</tr>
<tr>
<td>Recovery</td>
<td>Mean (%)</td>
<td>Range (%)</td>
<td>% Recovery after spiking</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>-----------</td>
<td>-------------------------</td>
<td></td>
</tr>
<tr>
<td>Serotonin Urine</td>
<td>96</td>
<td>74 - 105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>108</td>
<td>89 - 126</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Method comparison versus RIA**

| Urine | y = 0.94x + 19.58; R² = 0.98 |
| Serum | y = 0.85x + 33.18; R² = 0.97 |

*Commercially available RIA*

9. **References/Literature**


(3) Ciprandi et al. Serotonin in Allergic Rhinitis: a Possible Role for Behavioural Symptoms, 10(3):183-188 (2011)