TECHNICAL INFORMATION

Catalog Number: 193598
Serotonin - ELISA

Instructions for Use

Enzyme Immunoassay for the Quantitative Determination of Serotonin in Serum, Plasma, Urine, Tissue Homogenates, and Cell Culture Supernatants

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1. Introduction

Serotonin is an intermediate product of tryptophan metabolism and is located primarily in the enterochromaffin cells of intestine, serotoninergic neurons of the brain, platelets of the blood and is well established as a neurotransmitter in the central nervous system. Central serotoninergic neurons have been associated with physiological functions such as sleep, endocrine regulation, and cardiovascular control (1,2).

Nearly all of the serotonin in circulating blood is concentrated in platelets (13). Altered concentrations of circulating serotonin have been implicated in several pathological conditions including chronic tension headache (3,4), schizophrenia (5), hypertension (6,7), Huntington’s Disease (8), Duchenne’s muscular dystrophy (9), and early acute appendicitis (10). The determination of serum serotonin levels is of high clinical significance for diagnostic assessment of carcinoid syndrome (11).

2. Principle of the Test

The Serotonin-ELISA kit provides materials for the quantitative measurement of chemically derivatized serotonin in serum, plasma, urine, tissue homogenates, and cell culture supernatants. The sample preparation, i.e. derivatization of serotonin to N-acylserotonin, is part of the sample dilution and is achieved by incubation of the respective sample with the “Acylation Reagent”.

The assay procedure follows the basic principle of competitive ELISA whereby there is competition between a biotinylated and a non-biotinylated antigen for a fixed number of antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the free biotinylated antigen is removed by a washing step and the antibody bound biotinylated antigen determined by use of anti-biotin alkaline phosphatase as a marker and p-nitrophenyl phosphate as substrate. Quantification of unknowns is achieved by comparing the enzymatic activity of unknowns with a response curve prepared using known standards.

3. References

3.1 Product literature references


Address : Thomase Stratza, Hochrhein-Institut fur Rheumaforschung und Rheumaprevention, Bad Sackingen/Rheinfelden, Germany.

3.2. Other literature references


4. Precautions

– The assay calibrators and controls are of human origin and have been tested and confirmed negative for HIV I/II, HBsAg, and HCV by FDA approved procedures. All standards, however should be treated as potential biohazards in use and for disposal.
– The assay reagents contain sodium azide or thimerosal which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
– The stop solution contains NaOH. If it comes into contact with skin, wash thoroughly with water and seek medical attention. Since the NaOH used to terminate the color reaction is corrosive, the instrumentation employed to dispense it should be thoroughly cleaned after use.
– This kit is for in vitro diagnostic use only.
– Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
– Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
– Wear disposable latex gloves when handling specimens and reagents, and wash hands thoroughly afterwards. Microbial contamination of reagents or specimens may give false results.

5. Storage and Stability

The Serotonin-ELISA kit is shipped at ambient temperature and should be stored in the dark at 2-8°C. Do not use components beyond the expiration date shown on the kit labels. Do not mix various lots of any kit component within an individual assay.

6. Reagents Supplied

Sample Preparation:

6.1 Assay Buffer, Concentrate 1 bottle
50 ml, phosphate buffer with BSA and stabilizer, dilute 1:10 with distilled water before use.

6.2 Acylation Reagent 1 (3%) 1 vial
2 ml, ready to use, acetic acid anhydride in acetone.

6.3 Acylation Reagent 2 (10%) 1 vial
2 ml, ready to use, acetic acid anhydride in acetone.
**Enzyme Immunoassay:**

**6.4 Microtiter Strips**
12 break apart strips
each 8 wells
coated with anti-rabbit IgG from goat.

**6.5 Serotonin Biotin**
1 vial
6 ml, ready to use,
yellow colored

**6.6 Serotonin Antiserum**
1 vial
6 ml, ready to use,
antiserum from rabbit with stabilizers,
blue colored

**6.7 Enzyme Conjugate, Concentrate**
1 vial
0.2 ml,
anti-biotin antibody from goat, conjugated to
alkaline phosphate in Tris buffer with stabilizers,
dilute 1:100 with Assay Buffer.

**6.8 Standards A-G**
7 vials
0.5 ml each, ready to use,
contain acylated serotonin with stabilizers.

Concentrations:

<table>
<thead>
<tr>
<th>Standard</th>
<th>ABCDEFG</th>
<th>ng/ml</th>
<th>nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.05</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.14</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.41</td>
<td>2.37</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.2</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>3.7</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>11</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**6.9 Control Serum 1 and 2**
2 vials
lyophilized,
dissolve in 0.5 ml distilled water each,
for serotonin concentration see quality control
certificate. Store dissolved controls at -20°C or lower.

**6.10 Wash Buffer Concentrate**
1 bottle
50 ml,
phosphate buffer with Tween and stabilizer,
dilute 1:20 with distilled water.

**6.11 PNPP Substrate Tablets**
9 tablets
p-nitrophenyl phosphate (PNPP)

**6.12 PNPP Substrate Buffer**
1 vial
30 ml, ready for use,
contains diethanolamine.

**6.13 PNPP Stop Solution**
1 vial
10 ml, ready for use,
contains 1 N NaOH with 0.25 M EDTA.

**6.14 Adhesive Foil**
3 pieces

**Materials Required but not Supplied:**

- Pipettes 20, 25, 50, 100, and 1000 µl Multipette Eppendorf or similar products
- Disposable glass test tubes (12 x 75 mm)
- Polystyrene test tubes (12 x 75 mm)
- Vortex mixer
- Temperature controlled water bath, 37°C
- Centrifuge
- ELISA reader capable of reading absorbance at 405 nm

**7. Specimen Collection and Storage**

**Serum**
The usual precautions for venipuncture should be observed. Serum may be stored at room temperature for up to 3 hours, at
4-8°C for up to 12 hours, and should be frozen at -20°C or lower for longer periods.

**Urine**
The total volume of urine excreted during a 24 hour period should be collected and mixed in a single bottle containing 10-15 ml of 6 N hydrochloric acid as preservative. The exposure of samples to direct light should be avoided. Urine samples which are not assayed immediately may be stored at -20°C or lower for at least 6 months.

**Plasma samples and platelets**

More than 98% of the circulating serotonin is located in the platelets and is released during blood clotting (13). Blood must be collected by venipuncture into plastic tubes containing EDTA or Citrate as anticoagulant (e.g. 10 ml Monovette NC with 1 ml Citrate from SARSTEDT). Samples are kept and centrifuged at room temperature for 10 min at 200 xg to obtain platelet-rich plasma (PRP). The PRP-supernatant is then transferred to another tube and the platelets counted. An aliquot is further centrifuged at 450 xg for 10 min at 4°C to obtain platelet-free plasma (PFP).

To obtain the platelet pellet, an aliquot of 100 ml of PRP (containing between 350,000 and 500,000 platelets/ml) is added to 900 ml of physiological saline and centrifuged at 4500xg for 10 min at 4°C (or at 10,000xg for 2 min at 4°C). The supernatant is then discarded.

200 ml of double distilled water is added to the pellet, which can then be stored frozen at <-20°C for several weeks without any alteration of serotonin content.

After thawing of the frozen samples centrifuge at 10,000xg for 2 min at room temperature. 20 ml of the supernatant are used in the ELISA.

**Tissue Homogenates and Cell Culture Supernates**

Centrifuged tissue homogenates and cell culture supernates may be used without special precautions. Caution: Cell culture media may contain serotonin.

8. Preparation of Reagents

The contents of the kit can be divided into three separate runs. The volumes stated below are for one test procedure with 4 strips (32 determinations). If a larger number of strips is to be used, the volumes have to be changed accordingly.

8.1 Assay Buffer
15 ml of the Assay Buffer (concentrated) have to be diluted 1:10 with distilled water to make up 150 ml. The Assay Buffer is now ready for use. Store at 2-8°C.

8.2 Wash Buffer
Dilute 15 ml of the Wash Buffer Concentrate 1:20 with distilled water up to 300 ml. The Wash Buffer is now ready to use. Store at 2-8°C.

8.3 Control Sera
Add 0.5 ml of distilled water to each vial and let stand for 15 minutes. Mix by gently swirling. For longer storage, store in aliquots.

8.4 Anti-Biotin AP
Dilute 60 µl of the Enzyme Conjugate Concentrate with 6.0 ml of ready to use Assay Buffer. The anti-biotin AP is now ready to use. Prepare freshly prior to use.

8.5 PNPP Substrate Solution (should be prepared during the test procedure)
Dissolve 3 PNPP Substrate Tablets in 8 ml of PNPP Substrate Buffer. Dissolve the tablets approximately 10 minutes before the incubation with the Anti Biotin AP is finished.

9. Preparation of Samples

After removing assay reagents from the refrigerator, allow them to reach room temperature before pipetting. Unused reagents should be stored at 2 - 8°C. Sample preparation should be performed in glass tubes. Standards and unknowns should be assayed in duplicate.

**Acrylation of Samples and Controls**

The sample preparation (dilution and acylation) leads to a 207.25-fold (serum, urine, platelets, tissue homogenates, cell culture supernatants and a 23.5-fold (plasma) dilution of the samples, respectively. This has to be considered for the calculation of results.

1. Pipette 20 µl of sample (serum, urine, platelet-extract, tissue homogenate) and Control Sera, or 50 µl of platelet-free plasma (PFP) into glass test tubes.

2. Add 100 µl of ready to use Assay Buffer (ready to use) to each glass tube and vortex mix.

3. Add 25 µl of Acylation Reagent 1 (3%) for Serum, etc., or 25 µl of Acylation Reagent 2 (10%) for Plasma, to each glass tube. Vortex mix immediately.

4. Seal tubes and incubate for 15 min. at 37°C.

5. Add 4 ml (for Serum etc.), or 1 ml (for Plasma) of Assay Buffer. Vortex mix and remove the precipitated proteins by
9.6 Withdraw 50 µl aliquots for ELISA!

10. Assay Procedure ELISA

10.1 Pipette 50 µl each Standard, acylated Control Serum and acylated patient sample into the appropriate wells.

10.2 Pipette 50 µl of Serotonin-Biotin into each well.

10.3 Pipette 50 µl of Antiserum into each well and shake the plate carefully.

10.4 Seal the plate with the adhesive foil and incubate 16-20 hours (over night) at 2-8 °C.

10.5 Wash each well three times with Wash Buffer (the use of a washer is recommended!). Remove the Wash Buffer carefully. Invert plate and remove and tap firmly on clean blotting paper to remove any remaining liquid.

Note: Sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure.

10.6 Pipette 150 µl of Anti-Biotin AP into each well.

10.7 Seal with the adhesive foil and incubate for 120 min. at room temperature on an orbital shaker (500 rpm/1min).

Prepare the substrate solution 10 min. before end of incubation period!

10.8 Wash each well three times with Wash Buffer (see above).

10.9 Pipette 200 µl PNPP Substrate Solution into each well.

10.10 Incubate at room temperature for 60 min. on an orbital shaker (500 rpm/min).

10.11 Stop the substrate reaction by adding 50 µl PNPP Stop Solution into all wells. Briefly mix contents by gently shaking the plate.

10.12 Read the optical density at 405 nm (reference wave length 600-650 nm) with a microtiter plate reader within 60 min. after stopping.

11. Calculation of Results

The optical density of the substrate blank is subtracted from the optical densities of all standards and samples.

On a semi-logarithmic graph paper the concentration of the standards (abscissa, logarithmic) are plotted against their corresponding optical density (ordinate, linear). Alternately, the optical density of each standard and sample can be related to the optical density of the zero standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/ODmax, and then plotted on the ordinate. The concentration of the samples can be read directly from this standard curve by using their average optical density.

The sample preparation leads to a 207.25 fold dilution for Serum etc., or a 23.5 fold dilution for Plasma. The values read from the standard curve must be corrected accordingly:

Serum, Urine, Tissue Homogenates, and Cell Culture Supernates

Multiply by 207.25

Plasma (PFP)

Multiply by 23.5

This gives the serotonin concentration in ng/ml.

Below is listed a typical example of a standard curve with the Serotonin-Elisa.

<table>
<thead>
<tr>
<th>Concentration (nmol/l)</th>
<th>OD 1OD 2</th>
<th>Mean Value</th>
<th>OD/ODmax</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>0.082</td>
<td>0.020100</td>
<td>0.08</td>
<td>8.0</td>
</tr>
<tr>
<td>0.051</td>
<td>0.811</td>
<td>0.80089</td>
<td>10.1</td>
<td>1.0</td>
</tr>
<tr>
<td>0.141</td>
<td>5.881</td>
<td>6.251</td>
<td>6.0779</td>
<td>51.6</td>
</tr>
<tr>
<td>0.401</td>
<td>1.081</td>
<td>1.161</td>
<td>1.1255</td>
<td>0.05</td>
</tr>
<tr>
<td>1.200</td>
<td>6.200</td>
<td>6.500</td>
<td>6.3531</td>
<td>43.3</td>
</tr>
<tr>
<td>3.700</td>
<td>2.750</td>
<td>2.920</td>
<td>2.8414</td>
<td>0.42</td>
</tr>
<tr>
<td>11.00</td>
<td>1.340</td>
<td>1.396</td>
<td>1.9046</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Typical standard curve
Calculation of serotonin in platelets:

The content of serotonin in platelets is referred to $10^9$ platelets. Following is given an example.

Serotonin concentration: 50ng/ml;
Number of platelets in the PRP: 300.00/ml equivalent to 30,000,000/100ml PRP and 200ml of extraction volume. When using 20ml for the test this is a platelet equivalent of $3 \times 10^6$ platelets.

The serotonin content is referred to 1ml. Therefore the used platelets equivalent of 20ml has to be multiplied by 50. 
3 \times 10^6 = 50 \times 0.15 \times 10^9$ platelets/ml with a serotonin content of 50ng.

The resulting serotonin content in the platelets is 333ng/$10^9$ platelets ($50ng serotonin \times 1.0 \times 10^9 / 0.15 \times 10^9$).

12. Assay Characteristics

12.1 Expected values

12.1.1 Serum

It is recommended that each laboratory establish its own range of normal serotonin values. A normal range study was performed with this assay principle. The following are the results:

Serotonin reference values determined in human serum:

<table>
<thead>
<tr>
<th>n</th>
<th>34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mg/ml)</td>
<td>92.6</td>
</tr>
<tr>
<td>SD</td>
<td>49.7</td>
</tr>
<tr>
<td>Range (ng/ml)</td>
<td>up to 200</td>
</tr>
</tbody>
</table>

12.1.2 Platelet-free Plasma (PFP)

<p>| n | 35 |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (ng/ml)</strong></td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Range (ng/ml)</strong></td>
<td>1.8-7.5</td>
<td></td>
</tr>
</tbody>
</table>

### 12.1.3 Serotonin in platelets

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>35</td>
<td></td>
</tr>
<tr>
<td><strong>Mean (ng/10⁹ platelets)</strong></td>
<td>490</td>
<td></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>167</td>
<td></td>
</tr>
<tr>
<td><strong>Range (ng/10⁹ platelets)</strong></td>
<td>217-861</td>
<td></td>
</tr>
</tbody>
</table>

### 12.2 Specificity

The cross reactivity of the anti-N-acylserotonin antiserum has been measured against various compounds. In all cases, the interference from these compounds is unimportant due to the much higher levels of circulating serotonin. The percent cross reactivity is expressed as the ratio of serotonin concentration to the concentration of the reacting compound at 50% binding of the 0 ng/ml standard.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acylated Serotonin</td>
<td>100.0</td>
</tr>
<tr>
<td>Serotonin</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5-Hydroxytryptophol</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5-Hydroxy-3-indole acetic acid</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5-Hydroxytryptophan</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Melatonin</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

### 12.3 Sensitivity

The lowest detectable level that can be distinguished from the zero standard is 0.03 ng/ml (defined as 3 x standard deviation of the zero calibrator).

### 12.4 Precision

**INTRA-assay** variation (values in ng/ml)

<table>
<thead>
<tr>
<th>MeanStandardCV (%)n Deviation</th>
<th>11112.110.932</th>
</tr>
</thead>
<tbody>
<tr>
<td>41326.36.432</td>
<td></td>
</tr>
</tbody>
</table>

**INTER-assay** variation (values in ng/ml)

<table>
<thead>
<tr>
<th>MeanStandardCV (%)n Deviation</th>
<th>10614.313.410</th>
</tr>
</thead>
<tbody>
<tr>
<td>41230.57.410</td>
<td></td>
</tr>
</tbody>
</table>

### 12.5 Recovery
Normal human serum with a concentration of 0.09 µg/ml was enriched with increasing amounts of serotonin.

<table>
<thead>
<tr>
<th>Added (mg/ml)</th>
<th>Expected (mg/ml)</th>
<th>Actual (mg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.000</td>
<td>2.090</td>
<td>2.040</td>
<td>97.6</td>
</tr>
<tr>
<td>1.000</td>
<td>1.090</td>
<td>1.008</td>
<td>92.4</td>
</tr>
<tr>
<td>0.500</td>
<td>0.590</td>
<td>0.554</td>
<td>93.8</td>
</tr>
<tr>
<td>0.250</td>
<td>0.340</td>
<td>0.318</td>
<td>93.5</td>
</tr>
<tr>
<td>0.125</td>
<td>0.215</td>
<td>0.198</td>
<td>92.0</td>
</tr>
<tr>
<td>0.066</td>
<td>0.156</td>
<td>0.146</td>
<td>93.5</td>
</tr>
</tbody>
</table>

**13. Warranty**

Any modification of this test as well as exchanges or mixture of any components from different lots might influence the results. In such cases, there is no claim for a replacement.