Human Melatonin-Sulfate ELISA Kit

Cat. No.: DEIA2239
Pkg. Size: 96T

Intended use

This kit is the enzyme immunoassay for determination of melatonin sulfate (synonyms: 6-Hydroxymelatonin Sulfate, 6-Sulfatoxymelatonin) in human urine.

General Description

Melatonin also known chemically as N-acetyl-5-methoxytryptamine, is a naturally occurring compound found in animals, plants, and microbes. In animals, circulating levels of the hormone melatonin vary in a daily cycle, thereby allowing the entrainment of the circadian rhythms of several biological functions. Many biological effects of melatonin are produced through activation of melatonin receptors, while others are due to its role as a pervasive and powerful antioxidant, with a particular role in the protection of nuclear and mitochondrial DNA. Products containing melatonin have been available over-the-counter in the United States since the mid-1990s. In many other countries, the sale of this neurohormone is not permitted or requires a prescription.

Principle Of The Test

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed color is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

Reagents And Materials Provided

1. Microtiter Plate, Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal). Quantity: 1 x 12x8;
2. Melatonin Sulfate Antiserum; Ready to use. Contains: Antiserum (rabbit), Tris buffer, 0.01 % Thimerosal. Quantity: 1 x 5 mL;
3. Enzyme Conjugate Concentrate (40x) Contains: Melatonin Sulfate, conjugated to peroxidase, phosphate buffer 0.01 % Thimerosal. Quantity: 1 x 0.2 mL;
4. Standard A-G; Ready to use. 0; 1.7; 5.2; 15.6; 46.7; 140; 420 ng/mL; 0; 5.2; 15.9; 47.6; 142; 427; 1281 nmol/L; Contains: Melatonin Sulfate, Tris buffer, 0.01 % Thimerosal. Quantity: 1 x 7 x 0.1 mL;
5. Ready to use. Contains: 0.02 % Thimerosal. Quantity: 1 x 2 x 0.1 mL;
6. Assay Buffer, Ready to use. Red colored. Contains: Tris buffer, BSA, 0.01 % Thimerosal. Quantity: 1 x 60 mL;
7. Wash Buffer Concentrate (20x) Contains: phosphate buffer, Tween, 0.1 % Thimerosal. Quantity: 1 x 50 mL;
8. TMB Substrate Solution, Ready to use. Contains TMB, buffer, stabilizers. Quantity: 1 x 12 mL;
9. TMB Stop Solution, Ready to use. 1 M H2SO4. Quantity: 1 x 12 mL;
10. Adhesive Foil. Quantity: 3 x

Materials Required But Not Supplied

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 10; 50; 100; 1000 μL;
2. Round-bottom polystyrene test tubes (12 x 75 mm);
3. Rack for test tubes;
4. Orbital shaker (500 rpm);
5. Vortex mixer;
6. 8-Channel Micropipettor with reagent reservoirs;
7. Wash bottle, automated or semi-automated microtiter plate washing system;
8. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm);
9. Bidistilled or deionised water;

**Storage**

Store all contents at 2 to 8 °C.

**Specimen Collection And Handling**

Urine: It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle. Preservation is not necessary. Determine total volume for calculation of results.

**Reagent Preparation**

**Preparation of lyophilized or concentrated components**

1. Dilute 15mL wash buffer to 300mL with bidist water;  
   note: Resolve crystals at 18-25 °C and store at 2-8 °C;
2. Dilute enzyme conjugate with 2mL assay buffer.  
   note: Prepare freshly and use only once and store at 18-25 °C

**Dilution of Standards, Controls and Urine Samples**

1. Pipette 10 μL of each Standard, Control and urine sample into polystyrene, polypropylene or glass tubes. Avoid direct sunlight.
2. Pipette 500 μL of Assay Buffer into each tube. Vortex. Samples containing concentrations higher than the highest standard have to be further diluted with Assay Buffer.

**Assay Steps**

1. Pipette 50 μL of each diluted Standard, diluted Control and diluted sample into the respective wells of the Microtiter Plate;
2. Pipette 50 μL of freshly prepared Enzyme Conjugate into each well;
3. Pipette 50 μL of Melatonin Sulfate Antiserum into each well;
4. Cover plate with adhesive foil. Incubate 2 h at RT (18-25 °C) on an orbital shaker (500 rpm);
5. Remove adhesive foil. Discard incubation solution. Wash plate 4 x with 250 μL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel;
6. For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles;
7. Pipette 100 μL of TMB Substrate Solution into each well.
8. Incubate 30 min at RT (18-25 °C) on an orbital shaker (500 rpm);
9. Stop the substrate reaction by adding 100 μL of TMB Stop Solution into each well. Briefly mix contents by gently shaking the plate;
10. Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 60 min after pipetting of the Stop Solution;

**Quality Control**

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the
acceptable ranges as stated at the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

**Calculation**

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logistic or Logit-Log.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read from the standard curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in pre-test setup instructions and assayed.

**Typical Standard Curve**

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Melatonin Sulfate (ng/mL)</th>
<th>Mean OD</th>
<th>OD/ODₘₐₓ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0</td>
<td>1.805</td>
<td>100.0</td>
</tr>
<tr>
<td>B</td>
<td>1.7</td>
<td>1.741</td>
<td>96.5</td>
</tr>
<tr>
<td>C</td>
<td>5.2</td>
<td>1.536</td>
<td>85.1</td>
</tr>
<tr>
<td>D</td>
<td>15.6</td>
<td>1.185</td>
<td>65.7</td>
</tr>
<tr>
<td>E</td>
<td>46.7</td>
<td>0.773</td>
<td>42.8</td>
</tr>
<tr>
<td>F</td>
<td>140</td>
<td>0.341</td>
<td>18.9</td>
</tr>
<tr>
<td>G</td>
<td>420</td>
<td>0.164</td>
<td>9.1</td>
</tr>
</tbody>
</table>

**Precautions**

1. Avoid contact with Stop solution. It may cause skin irritations and burns.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood;
3. Don't use damaged components in test runs, but keep safe for complaint related issues;
4. Obey lot number and expiry date. Don't mix reagents of different lots. Don't use expired reagents;
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary;
REFERENCES


