MELATONIN PLASMA RIA

$^{125}$I-Radioimmunoassay for the
Quantitative Determination of Melatonin in Serum and Plasma

- For research use only -

Melatonin Plasma RIA (100 Determinations)  
Cat. No.: 07L-107102

Store at 2 – 8 °C
I. PRECAUTIONS

Safety Rules And Regulations

This kit contains test components produced with parts of human tissues. There is no known test method which definitively precludes the transmission of hepatitis, HIV or other viral infections through products of these cells. It is therefore recommended that the components of the kit as well as those for specific samples are treated as potentially infectious. It is probable that antibodies against the hepatitis C virus can be found in the product. Although the existence of HCV antibodies does not necessarily mean that the materials are infectious, suitable precautions should be taken during handling to prevent any risk of infections.

Never pipette by mouth!

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 hands thoroughly as soon as you have finished the work.

Avoid spraying of any kind!

Remove the samples and decontaminate each and every material if there is the slightest chance that it might be contaminated. The preferred method of decontamination is autoclaving for at least one hour at 121°C.

Sodium azide can react to lead and copper tubes and form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.

This radioactive product may only be handed over to persons or institutions or acquired by those unless they have the necessary and respective authority for handling, storage or use. The components of the kit are intended exclusively for in-vitro diagnosis.

The acquisition, storage, use and disposal of radioactive material are specified by the radiation protection directive.

A. Summary of the basic principles of the radiation protection directive:

1. Radioactive material may only be stored in its original packing and reserved spaces.
2. Receipt, storage and use of this material must be recorded.
3. Radioactive material may only be handled and used in rooms specified for this purpose (controlled area).
4. Avoid any direct contact with radioactive material by wearing protective clothing and protective gloves.
5. To prevent cross-contamination with other isotopes, ensure that contaminated laboratory equipment and glasses are disposed of as directed.
6. Use the required care and attention to clear up any contamination.
7. Disposal of radioactive material must be carried out in accordance with the respective current directive.

B. User Rules And Regulations

1. Do not use any components of the kit if they are past the use-by date.
2. Do not mix up test reagents of different charges!
3. Avoid microbial contamination of the test reagents and washing water.
4. Observe the incubation periods and washing instructions.

II. GENERAL INFORMATION

A. Introduction And Principle Of The Test

Melatonin - the major hormone secreted by the pineal gland - is a key modulator of annual and circadian biorhythms. Its circadian profile in body fluids is an excellent marker for the setting of the endogenous clock. Daytime plasma Melatonin levels are low and rise in the evening (onset). Night-time levels peak at around 03.00 hrs. (acrophase) in most healthy humans.

As a general modulator of human biorhythm, Melatonin is involved in the timing of functions such as sleep, mood, reproduction and immune system activity.

The phase of the Melatonin profile may be shifted in conditions such as sleep disorders, seasonal affective disorders, depression or menstrual dysregulation.

Onset, acrophase and offset have a stable phase relationship even when the phase of the Melatonin profile is shifted.

The assay kit provides materials for the quantitative measurement of Melatonin in plasma and serum.
The 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-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When 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 unknown standards.

III. STORAGE AND STABILITY

The reagents should be stored 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.

IV. CONTENTS OF THE KIT

| BA 0801 | Standard A | 0 pg/mL (Melatonin) The lot specific concentration is indicated on the label of the vial and in the QC Report | 1 x 2 mL, lyophilized, redissolve in 2 mL dist. Water |
| BA 0802 | Standard B | 1.25 pg/mL (Melatonin) The lot specific concentration is indicated on the label of the vial and in the QC Report | 1 x 1 mL, lyophilized, redissolve in 1mL dist. Water |
| BA 0803 | Standard C | 5 pg/mL (Melatonin) The lot specific concentration is indicated on the label of the vial and in the QC Report | 1 x 1 mL, lyophilized, redissolve in 1mL dist. Water |
| BA 0804 | Standard D | 20 pg/mL (Melatonin) The lot specific concentration is indicated on the label of the vial and in the QC Report | 1 x 1 mL, lyophilized, redissolve in 1mL dist. Water |
| BA 0805 | Standard E | 80 pg/mL (Melatonin) The lot specific concentration is indicated on the label of the vial and in the QC Report | 1 x 1 mL, lyophilized, redissolve in 1mL dist. Water |
| BA 0806 | Standard F | 320 pg/mL (Melatonin) The lot specific concentration is indicated on the label of the vial and in the QC Report | 1 x 1 mL, lyophilized, redissolve in 1mL dist. Water |
| BA 0851 | Control 1 | 1 x 1 mL, lyophilized, redissolve in 1mL dist. Water |
| BA 0852 | Control 2 | 1 x 1 mL, lyophilized, redissolve in 1mL dist. Water |
| BA 0813 | Assay Buffer | 1 x 6 mL, ready for use, Caution, corrosive liquid! |
| BA 0815 | Enzyme | 2 x 3 mL/vial, lyophilized, redissolve each in 3 mL Enzyme Buffer |
| BA 0816 | Enzyme Buffer | 2 x 4 mL, ready for use, Caution, corrosive liquid! |
| BA 0810 | Melatonin Antiserum | 1 x 5,25 mL, from rabbit, ready for use, blue coloured |
| BA 0820 | $^{125}$I – Melatonin Tracer | 1 x 5.5 mL, activity < 200 kBq, ready for use, red coloured |
| BA 3030 | Precipitating Reagent 55 mL, ready for use, goat anti-rabbit serum in PEG phosphate buffer. Mix thoroughly before use! |

V. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

A. Automatic pipettes for 25, 50, 100 and 500 µL
B. Polystyrene tubes and suitable rack
C. Centrifuge capable of at least 3,000 x g
D. Suitable device for aspirating or decanting the tubes
E. Vortex mixer
F. Gamma counter
G. Distilled water
VI. SAMPLE COLLECTION AND STORAGE

The test can be performed with EDTA plasma as well as with heparin plasma and serum. Haemolytic and especially
lipemic samples should not be used for the assay, because false low values will be obtained with such samples. The
plasma samples can be stored up to 24 hours at 2 - 8°C. For a longer period (up to 6 months) the samples should
be stored at -20°C.

Repeated freezing and thawing should be avoided.

VII. TEST PROCEDURE

A. Preparation of Reagents

Plasma Standards

Reconstitute Standard A with 2 mL distilled water, Standards B - F each with 1 mL. Reconstituted standards
which are not used immediately have to be frozen at -20 °C (in aliquotes) and may be thawed only once.

Controls 1 & 2

Reconstitute the controls each with 1 mL distilled water. Reconstituted controls which are not used
immediately have to be frozen at -20 °C (in aliquotes) and may be thawed only once.

Enzyme

Reconstitute the content of the vial with 3 mL Enzyme Buffer prior to use. Mix carefully (30 minutes on a
rotating mixer). The reconstituted enzyme cannot be stored and has to be used only once. Upon request
further Enzyme vials are provided.

B. Melatonin RIA (Plasma and Serum)

Allow reagents and samples to reach room temperature. Number the assay tubes (polystyrene). Duplicates
are recommended.

1. Pipet 100 µL of Standard A into the tubes for the NSB.
2. Pipet 100 µL of Standards A - F, controls and patient sample into the respective tubes.
3. Pipet 25 µL of Enzyme solution in all tubes (except Totals), mix shortly and centrifuge 1 minute at
   500 x g.
4. Incubate for 1 hour at room temperature (approx. 20°C).
5. Pipet 50 µL of Assay-Buffer into all tubes (except Totals) and mix shortly.
6. Pipet 50 µL of $^{125}$I-Tracer into all tubes.
7. Pipet 50 µL of Antiserum into all tubes (except Totals and NSB).
8. Mix thoroughly and centrifuge for 1 minute at 500 x g.
9. Incubate for 15 to 20 hours at room temperature (approx. 20°C).
10. Pipet 500 µL of the cold (2 - 8 °C) Precipitating Antiserum into all tubes (except Totals) and mix.
11. Incubate for 15 minutes at 2 - 8 °C.
12. Centrifuge for 15 minutes at 3,000 x g (preferably refrigerated).
13. Decant or aspirate the supernatant carefully (except Totals).
14. Count all tubes for 1 minute in a gamma counter.
VIII. CALCULATION OF RESULTS

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of Zero Reference (=Standard A), Standards B - F, Control 1 & 2 and patient samples. Construct a standard curve by plotting the percentage of cpm of each Standard in relation to the cpm of the Zero Reference (B-NSB/B0-NSB in %) versus its corresponding concentration. The concentration of the Controls and patient samples can then be read off the standard curve by using their percentage of B-NSB/B0-NSB.

A. Quality Control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The ICN or other commercial controls should fall within established confidence limits. The confidence limits of the ICN Controls are printed on the Control vial labels.

B. Quantitative

The calibration curve from which the concentration of Melatonin in the samples can be taken is obtained by plotting % B/B₀ values measured for the 6 standards (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis). The results for unknowns can be calculated using one of the following curve-fitting techniques: spline fits, Akima or four-parameter logistic. The following plots are examples of typical calibration curve for the Melatonin RIA. Please do not use this curve for the determination of Melatonin in samples.
IX. ASSAY CHARACTERISTICS

A. Specificity

Various substances were tested for possible interference in the assay. Listed below are the cross reactivities of the selected compounds with the antiserum against Melatonin. Given are the ratios of the molar concentration of Melatonin to the molar concentration of the examined compound at 50% displacement of the Melatonin tracer.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin</td>
<td>100.00</td>
</tr>
<tr>
<td>N-Acetylserotonin</td>
<td>0.80</td>
</tr>
<tr>
<td>5-Methoxytryptophol</td>
<td>0.70</td>
</tr>
<tr>
<td>5-Methoxytryptamine</td>
<td>0.08</td>
</tr>
<tr>
<td>6-Methoxytryptamine</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>5-Methoxyindol-3-acetic acid</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Serotonin</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DL-Tryptophan</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DL-5-Methoxytryptophan</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>5-Hydroxy-L-Tryptophan</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

B. Reference Ranges

The reference concentrations given below should be taken as a guideline only. It is recommended that each laboratory should establish its own normal values.

The Melatonin concentrations depend on age and on a circadian rhythm with a maximum at night between 1.00 and 3.00 a.m. This maximum is usually clearly higher than the values during the daytime. The highest concentrations are found with infants up to the age of 3 years.

Healthy adults show melatonin concentrations up to 15 pg/ml in plasma and serum during the daytime.

C. Sensitivity

The lower limit of detection was determined by taking the 3-fold standard deviation of the cpm of the Zero Reference and reading the corresponding value from the standard curve. For plasma and serum it is 0.75 pg/mL.

D. Reproducibility

The reproducibility of the RIA tests was investigated by determining the intra- and inter-assay coefficients of variation (CV) by repeated measurements of two plasma samples with different Melatonin concentrations.
Melatonin RIA:

### Intra-Assay Variation, n = 30

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.76 ± 0.28</td>
<td>7.4</td>
</tr>
<tr>
<td>2</td>
<td>60± 2.6</td>
<td>4.3</td>
</tr>
</tbody>
</table>

### Inter-Assay Variation, n = 6

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean ± SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.3 ± 1.72</td>
<td>12.1</td>
</tr>
<tr>
<td>2</td>
<td>3.2 ± 0.38</td>
<td>11.7</td>
</tr>
</tbody>
</table>

X. PIPETTING SCHEME

<table>
<thead>
<tr>
<th>T</th>
<th>NSB</th>
<th>B₅</th>
<th>Standards</th>
<th>Controls</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Melatonin:</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Standard A</td>
<td>100 µL</td>
<td>100 µL</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Standard B - F</td>
<td>100 µL</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Controls</td>
<td>100 µL</td>
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<td></td>
<td></td>
<td></td>
<td>Samples</td>
<td>100 µL</td>
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<td></td>
<td></td>
<td></td>
<td>Enzyme solution</td>
<td>25 µL</td>
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<td></td>
<td>25 µL</td>
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<td>25 µL</td>
<td>25 µL</td>
</tr>
</tbody>
</table>

Mix thoroughly
Centrifuge for 1 min. at 500 x g
Incubate for 1 h at room temperature

<table>
<thead>
<tr>
<th>Assay Buffer</th>
<th>50 µL</th>
<th>50 µL</th>
<th>50 µL</th>
<th>50 µL</th>
<th>50 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>^125^I-Tracer</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
<tr>
<td>Antiserum</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

Mix thoroughly
Centrifuge for 1 minute at 500 x g
Incubate for 15 - 20 hours at room temperature

| Prec. Reagent (cool 2-8°C) | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |

Incubate for 15 minutes at 2–8°C
Centrifuge for 15 minutes at least 3000 x g (preferably cool)
Decant or aspirate supernatant carefully (except T)
Count all tubes for 1 minute in a gamma counter
XI. REFERENCES


Manufactured by: ICN Pharmaceuticals, Inc.
Hergestellt von: Diagnostics Division
Fabriqué par: 13 Mountain View Avenue
Fabbricato da: Orangeburg, New York 10962-1294
Fabricado por: http://www.icndiagnostics.com

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