

Manufactured for:

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## Instructions for use

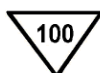
# Melatonin Direct RIA

<sup>125</sup>I – Radioimmunoassay for the direct determination of Melatonin in human serum, plasma and saliva.

**For Research Use Only, Not for Use in Diagnostic Procedures.**

**REF**

**IB88111**



**RUO**

For Research use only-  
Not for use in diagnostic  
procedures

**< 200 KBq**  
**< 5.40 µCi**

## Melatonin RIA

### 1. **Intended use**

<sup>125</sup>I – Radioimmunoassay for the direct determination of Melatonin in human serum, plasma and saliva. For research use only, not for use in diagnostic procedures.

### 2. **Principle of the test**

The assay procedure follows the basic principle of radioimmunoassay, involving competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of <sup>125</sup>I-labelled antigen bound to the antibody is inversely proportional to the analyte concentration of the unknown. 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. Determination of unknowns is achieved by comparing their activity with a reference curve prepared with known calibrators.

### 3. **Procedural Notes**

#### 3.1 **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.

#### 3.2 **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 federation 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 safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

#### 3.3 **Interference**

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of the biological being tested 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.

#### 3.4 **Precautions**

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and biologicals with skin. No smoking, eating or drinking in areas where biologicals or kit test tubes are handled. When working with kit components or unknowns, always wear protective gloves and wash your hands 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 <sup>125</sup>Iodine (half life: 60 days), emitting ionizing X- (28 keV) and G- (35.5 keV) radiations.

The receipt, acquisition, possession, use, and transfer of radioactive materials are subject to license and regulations of the U.S. Nuclear Regulatory Commission or the State with which the NRC has entered into an agreement for the exercise of regulatory authority. Radioactive material may be received, acquired, possessed, and used only by qualified authorized personnel and only for laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals.

All radioactive material should be stored in specially designated areas. Handling and use of radioactive materials should be done only in designated areas by authorized personnel. Any radioactive spills must be cleaned immediately and all contaminated materials disposed of as radioactive waste in accordance with the appropriate regulatory authority and radio safety procedures. Adherence to the basic rules of laboratory and radiation safety provides adequate protection.

All reagents of this test kit 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.

#### 4. **Storage and stability**

The reagents should be stored at 2 - 8 °C until expiration date. 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. Melatonin is sensitive to light-exposure. To avoid photo-oxidative reduction of melatonin, it is necessary to keep it away from direct sun light and from heat.

#### 5.1 **Contents of the kit**

<b>BA R-0028</b>	EQUA-REAG	<b>Equalizing Reagent</b>	2 x 10 mL	lyophilized
<b>BA R-0030</b>	PREC-REAG	<b>Precipitating Reagent</b>	2 x 55 mL	ready for use, goat anti-rabbit serum in PEG phosphate buffer, yellow screw cap. <i>Mix thoroughly before use!</i>
<b>BA R-3301</b>	CALIBRATOR A	<b>Calibrator A</b>	1 x 4 mL	ready for use
<b>BA R-3302</b>	CALIBRATOR B	<b>Calibrator B</b>	1 x 4 mL	ready for use
<b>BA R-3303</b>	CALIBRATOR C	<b>Calibrator C</b>	1 x 4 mL	ready for use
<b>BA R-3304</b>	CALIBRATOR D	<b>Calibrator D</b>	1 x 4 mL	ready for use
<b>BA R-3305</b>	CALIBRATOR E	<b>Calibrator E</b>	1 x 4 mL	ready for use
<b>BA R-3306</b>	CALIBRATOR F	<b>Calibrator F</b>	1 x 4 mL	ready for use
<b>BA R-3307</b>	CALIBRATOR G	<b>Calibrator G</b>	1 x 4 mL	ready for use
<b>BA R-3310</b>	AS MEL	<b>Melatonin Antiserum</b>	1 x 5.25 mL	from rabbit, ready for use, blue coloured, blue screw cap
<b>BA R-3313</b>	ASSAY-BUFF	<b>Assay Buffer</b>	1 x 15 mL	ready for use
<b>BA R-3315</b>	ENZYME	<b>Enzyme</b>	4 x 3 mL	lyophilized
<b>BA R-3316</b>	ENZYME-BUFF	<b>Enzyme Buffer</b>	1 x 15 mL	ready for use
<b>BA R-3320</b>	<sup>125</sup> I-MEL	<b><sup>125</sup>I – Melatonin</b>	1 x 5.5 mL	activity < 200 kBq, ready for use, red coloured, red screw cap
<b>BA R-3351</b>	CONTROL 1	<b>Control 1</b>	1 x 4 mL	ready for use
<b>BA R-3352</b>	CONTROL 2	<b>Control 2</b>	1 x 4 mL	ready for use

Calibrator	Melatonin concentration of the calibrators refer to:						
	A	B	C	D	E	F	G
Serum, EDTA-Plasma (pg/mL)	0	3	10	30	100	300	1,000
Serum, EDTA-Plasma (pmol/L)	0	12.9	43	129	430	1,290	4,300
Saliva (pg/mL)	0	1	3	10	30	100	300
Saliva (pmol /L)	0	4.3	12.9	43	129	430	1,290
<b>Conversion:</b>	<b>Melatonin (pg/mL) x 4.30 = Melatonin (pmol/L)</b>						

#### 5.2 **Additional materials and equipment required but not provided with the kit**

- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000 µL)
- Conical plastic tubes (polypropylene, polystyrene) and suitable rack
- 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

#### 6. **Collection and Handling of Unknowns**

##### **Serum and EDTA Plasma**

The test can be performed with EDTA plasma as well as with serum.

Haemolytic and especially lipemic biologicals should not be used with this assay. The unknowns can be stored for up to 24 hours at 2 - 8 °C. For a longer period (up to 6 months) they should be stored at -20 °C. Repeated freezing and thawing should be avoided.

##### **Saliva**

Saliva has to be collected in adequate collection devices. Eating, drinking, and brushing teeth should be avoided for at least 30 min. before collecting. Saliva should be collected without stimulation and be frozen at -20 °C immediately. After thawing the saliva has to be centrifuged for 5 minutes (2,000 - 3 000 x g) immediately before the performance of the RIA. Repeated freezing and thawing should be avoided.

## 7. 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 accordingly. Duplicate determinations are recommended.

⚠ *Pipetted liquids should not adhere to the wall of the RIA tubes. If necessary please centrifuge the tubes for 1 minute at 500xg to spin down adhering liquids.*

⚠ *The use of conical tubes is highly recommended for the assay.*

### 7.1 Preparation of reagents

#### Enzyme

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

#### Equalizing Reagent

The Equalizing Reagent has to be reconstituted with 10 mL distilled water. Reconstituted Equalizing Reagent which is not used immediately has to be frozen at -20 °C (in aliquots) and may be thawed only once.

### 7.2 Melatonin RIA (Serum, EDTA-Plasma)

1.	Pipette <b>20 µL</b> of <b>Calibrator A</b> into the tubes for the <b>NSB</b> and into the tubes for the <b>unknowns</b> .
2.	Pipette <b>20 µL</b> of <b>calibrators</b> and <b>controls</b> into the respective tubes.
3.	Add <b>200 µL</b> of <b>Equalizing Reagent</b> to the tubes with <b>NSB, calibrators</b> and <b>controls</b> .
4.	Pipette <b>200 µL</b> of the <b>unknowns</b> into the respective tubes.
5.	Pipette <b>50 µL</b> of <b>Enzyme</b> (refer to 7.1) in all tubes ( <b>except totals</b> ) and vortex.
6.	Incubate for <b>1 hour</b> at <b>RT</b> (20-25 °C).
7.	Pipette <b>100 µL</b> of Assay Buffer into all tubes (except totals) and mix shortly.
8.	Pipette <b>50 µL</b> of <b>Melatonin Antiserum</b> into <b>all tubes (except totals and NSB)</b> ; mix thoroughly.
9.	Incubate for <b>1 hour</b> at <b>RT</b> (20-25 °C).
10.	Pipette <b>50 µL</b> of the <b><sup>125</sup>I Melatonin</b> into <b>all tubes</b> and mix thoroughly.
11.	Cover tubes. Incubate for <b>20 - 24 hours</b> at <b>RT</b> (20-25 °C).
12.	Mix the chilled (2 - 8 °C) <b>Precipitating Reagent</b> thoroughly, pipette each <b>1000 µL</b> into <b>all tubes (except totals)</b> , and mix on a vortex.
13.	Incubate for <b>20 minutes</b> at <b>2 - 8 °C</b> .
14.	Centrifuge for <b>20 minutes</b> at <b>3 000 x g</b> , if possible in a refrigerated centrifuge.
15.	<b>Decant</b> or aspirate the <b>supernatant carefully (except totals)</b> . Blot the tubes dry and leave them upside for 2 minutes.
16.	<b>Count</b> all tubes for <b>1 minute</b> in a gamma counter.

### 7.3 Melatonin RIA (Saliva)

1.	Pipette <b>20 µL</b> of <b>Calibrator A</b> into the tubes for the <b>NSB</b> and into the tubes for the <b>unknowns</b> .
2.	Pipette <b>20 µL</b> of <b>calibrators</b> and <b>controls</b> into the respective tubes.
3.	Add <b>600µL</b> of <b>distilled water</b> to the tubes for <b>NSB, calibrators</b> and <b>controls</b> .
4.	Pipette <b>600 µL</b> of the <b>unknowns</b> into the respective tubes.
5.	Pipette <b>25 µL</b> of <b>Enzyme</b> (refer to 7.1) into all tubes ( <b>except totals</b> ) and vortex.

<b>6.</b>	Incubate for <b>1 hour</b> at <b>RT</b> (20-25 °C).
<b>7.</b>	Pipette <b>50 µL</b> of Assay Buffer into all tubes (except totals) and mix shortly.
<b>8.</b>	Pipette <b>20 µL</b> of <b>Melatonin Antiserum</b> into <b>all tubes</b> ( <b>except totals and NSB</b> ); mix thoroughly.
<b>9.</b>	Incubate for <b>1 hour</b> at <b>RT</b> (20-25 °C).
<b>10.</b>	Pipette <b>50 µL</b> of the <b><sup>125</sup>I Melatonin</b> into <b>all tubes</b> and mix thoroughly.
<b>11.</b>	Cover the tubes. Incubate for <b>20 - 24 hours</b> at <b>RT</b> (20-25 °C).
<b>12.</b>	Mix the chilled (2 - 8 °C) <b>Precipitating Reagent</b> thoroughly, pipette each <b>1 mL</b> into <b>all tubes</b> ( <b>except totals</b> ), and mix on a vortex.
<b>13.</b>	Incubate for <b>20 minutes</b> at <b>2 - 8 °C</b> .
<b>14.</b>	Centrifuge for <b>20 minutes</b> at <b>3 000 x g</b> , if possible in a refrigerated centrifuge.
<b>15.</b>	<b>Decant</b> or aspirate the <b>supernatant</b> <u>carefully</u> ( <b>except totals</b> ). Blot the tubes dry and leave them upside for 2 minutes.
<b>16.</b>	<b>Count</b> all tubes for <b>1 minute</b> in a gamma counter.

## 8. Results

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of calibrators, controls and unknowns.

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

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

### **Serum/ Plasma and controls:**

The concentrations of the **serum and plasma** and the **controls** can be read directly from the calibration curve Melatonin (**Serum**).






### **Saliva and controls:**

The concentrations of the **saliva** and the **controls** can be read directly from the Melatonin Saliva calibration curve.

### 8.1 **Quality control**

It is recommended to use controls according to state and federal regulations. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

### **Symbols:**

	Storage temperature			Contains sufficient for <n> tests
	Expiry date	<b>LOT</b>	Batch code	<b>IVD</b>
	Consult instructions for use	<b>CONT</b>	Content	<b>CE</b>
	Caution	<b>REF</b>	Catalogue number	<b>RUO</b>
				For research use only!

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