DIAsource ImmunoAssays S.A.
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Belgium

Instructions for use

Melatonin Direct RIA

REF KIPL3300

100

+2 +8 °C

IVD

CE

< 200 KBq
< 5.40 µCi

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the direct quantitative determination of Melatonin in human serum and plasma.

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.
Onset, acrophase and offset have a stable phase relationship even when the phase of the melatonin profile is shifted.
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 125I-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 known standards.

2. Advice on handling the test

2.1 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.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 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.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples 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.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. 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 hand 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.

The radioactive material may be received, acquired, possessed, and used only by physicians, veterinarians in the practice of veterinary medicine, clinical laboratories or hospitals and only for in vitro clinical or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority. In no case the product must be administered to humans or animals.
All radioactive handling should be executed in a designated area, away from regular passage. A log book for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radio safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

3. 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.

4.1 Contents of the kit

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

4.2 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**

5. Sample collection and storage

Serum and EDTA Plasma
The test can be performed with EDTA plasma as well as with serum.
Haemolytic and especially lipemic samples should not be used with this assay, because false low values will be obtained with such samples. The samples can be stored for 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.

6. 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.***

6.1 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.

6.2 Melatonin RIA

1.	Pipette 15 µL of standards and controls into the respective tubes.
2.	Pipette 150 µL of Equalizing Reagent into the tubes for NSB, standards and controls.
3.	Pipette 150 µL of the samples into the respective tubes.
4.	Add 50 µL of Enzyme (refer to 6.1*)* to all tubes *(except totals)* and vortex.
5.	Incubate for 1 hour at RT (20-25 °C).
6.	Pipette 100 µL of Assay Buffer into all tubes (except totals) and mix shortly.
7.	Pipette 25 µL of the ^{125}I Melatonin into all tubes.
8.	Pipette 50 µL of Melatonin Antiserum into all tubes (*except totals and NSB*); mix thoroughly.
9.	Cover tubes. Incubate for 20 - 24 hours at RT (20-25 °C).
10.	Mix the chilled (2 - 8 °C) Precipitating Reagent thoroughly, pipette each 1000 µL into all tubes (*except totals*), and mix on a vortex.
11.	Incubate for 20 minutes at 2 - 8 °C.
12.	Centrifuge for 20 minutes at 3 000 x g, if possible in a refrigerated centrifuge.
13.	Decant or aspirate the supernatant carefully (*except totals*). Blot the tubes dry and leave them upside for 2 minutes.
14.	Count all tubes for 1 minute in a gamma counter.

7. Calculation of results

	Melatonin concentration of the standards refer to:						
Standard	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
Conversion:	Melatonin (pg/mL) x 4.30 = Melatonin (pmol/L)						

Subtract the mean cpm of the non-specific binding NSB from the mean cpm of standards, controls and samples.
The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the percentage of (B-NSB)/(B0-NSB) measured for 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).

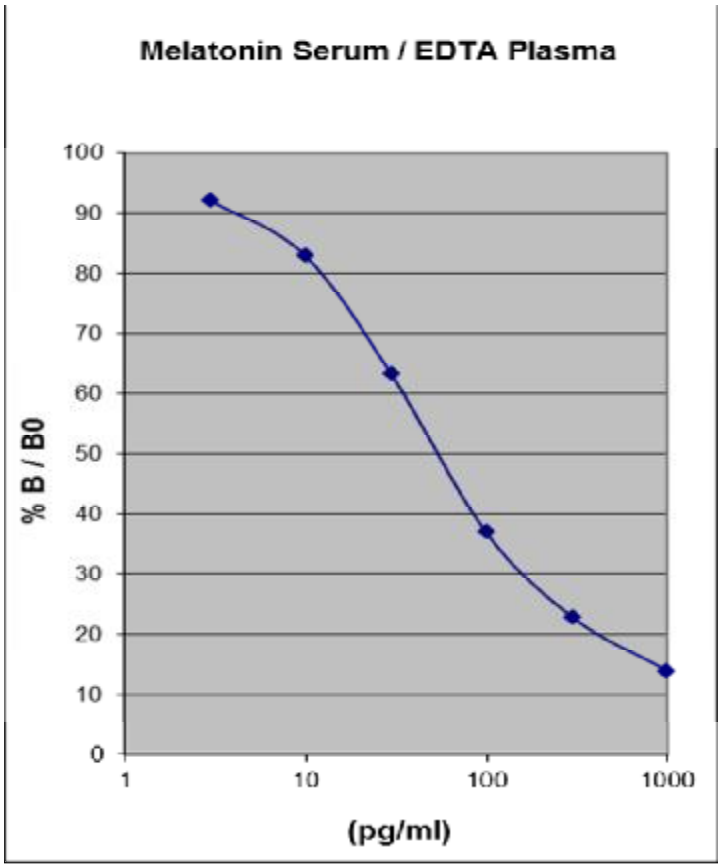
Serum / Plasma samples and controls:
The concentrations of the serum and plasma samples and the controls can be read directly from the calibration curve Melatonin (Serum).

7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-report.

7.2 Typical calibration curves

⚠ ***Examples, do not use for calculation!***



8. Assay characteristics

Expected Reference Values	**Serum, EDTA-Plasma** **The reference concentration 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 serum and plasma melatonin levels in humans show a marked circadian rhythm characterized by very low levels during day time (up to 30 pg/mL for serum/ plasma) and high levels during night time (up to 150 pg/mL for serum/ plasma). The highest concentrations are found with infants up to the age of 3 years.**

Analytical Sensitivity (Limit of Detection)	**Melatonin Serum**
	2.3 pg/mL

	Substance	**Cross Reactivity (%)**
		Melatonin
Analytical Specificity (Cross Reactivity)	**Melatonin**	**100**
	N-Acetylserotonin	**0.98**
	5-Methoxytryptophol	**0.11**
	5-Methoxytryptamine	**0.07**
	6-Methoxytryptamine	**< 0.01**
	5-Methoxyindol-3-acetic acid	**< 0.01**
	Serotonin	**< 0.01**
	DL-Tryptophan	**< 0.01**
	DL-5-Methoxytryptophan	**< 0.01**
	5-Hydroxy-L-Tryptophan	**< 0.01**

Precision							
Intra-Assay				**Inter-Assay**			
	Sample	**Range (pg/mL)**	**CV (%)**		**Sample**	**Range (pg/mL)**	**CV (%)**
Melatonin Serum/ Plasma	**1**	**19.2 ± 1.9**	**9.8**	**Melatonin Serum/ Plasma**	**1**	**29.4 ± 2.4**	**8.0**
	2	**41.8 ± 4.0**	**9.7**		**2**	**73.9 ± 8.1**	**10.9**
	3	**126 ± 16.9**	**13.4**		**3**	**154 ± 20.6**	**13.3**

Linearity		**Range**	**Mean (%)**	**Range (%)**
	Melatonin Serum / Plasma	**8.5 – 529 pg/ mL**	**89**	**82 – 102**

Recovery		**Range**	**Mean (%)**	**% Recovery after spiking**
	Melatonin Serum	**19.7 – 808 pg/ mL**	**90**	
	Melatonin Plasma	**19.4 – 529 pg/ mL**	**84**	

Stability (Freeze and Thaw Stability)	**Stability (comparison fresh sample with freeze and thaw stability)**						
	Sample	**Fresh Sample**	**Freeze and Thaw Stability**	**Mean (pg/mL)**	**Deviation (%)**	**SD (pg/mL)**	**CV (%)**
	1	**21.4 pg/mL**	**30.8 pg/mL**	**26.1**	**43.9**	**6.6**	**25.4**
	2	**39.4 pg/mL**	**50.4 pg/mL**	**44.9**	**27.9**	**7.8**	**17.3**
	3	**205.4 pg/mL**	**190.9 pg/mL**	**198.2**	**7.1**	**10.3**	**5.2**
	Stability (comparison fresh sample with short-term temperature stability)						
	Sample	**Fresh Sample**	**Short-Term Temp. Stability**	**Mean (pg/mL)**	**Deviation (%)**	**SD (pg/mL)**	**CV (%)**
	1	**21.4 pg/mL**	**31.4 pg/mL**	**26.4**	**46.7**	**7.1**	**26.9**
	2	**39.4 pg/mL**	**50.3 pg/mL**	**44.9**	**27.7**	**7.7**	**17.2**
	3	**205.4 pg/mL**	**209.5 pg/mL**	**207.5**	**2.0**	**2.9**	**1.4**

⚠ ***For updated literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	**Storage temperature**		**Manufacturer**		**Contains sufficient for <n> tests**
	**Expiry date**	LOT	**Batch code**	I V D	**For in-vitro diagnostic use only!**
	**Consult instructions for use**	CONT	**Content**	CE	**CE labelled**
	**Caution**	REF	**Catalogue number**	RUO	**For research use only!**