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LDN®

Instructions for use

Melatonin Saliva Direct RIA

REF **BA R-3400**

IVD CE 200 kBq

Melatonin Saliva RIA

1. Intended use and principle of the test

^{125}I – Radioimmunoassay for the direct quantitative determination of Melatonin in saliva.

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 saliva 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 ^{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 known standards.

2. Precautions, Guidelines and Warnings

- This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Reagents of this kit which contain human 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.
- The principles of Good Laboratory Practice (GLP) have to be followed.
- In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- The radioactive material (^{125}Iodine, half life 60 days, emitting ionizing X-radiation with 28 kev and G-radiation with 35.5 kev) may be received, acquired, possessed and used only by physicians, laboratories or hospitals. In compliance with regulations, a copy of the customer's current radioisotope license must be on file with the supplier. Orders cannot be shipped until the license is received by the supplier (Radiation Protection Act of June 30, 1989).
- For the dilution or reconstitution purposes use deionized, distilled, or ultra-pure water.
- Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Incubation times do influence the results. All tubes should be handled in the same order and time intervals.
- To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- A calibrator curve must be established for each run.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, rinse off immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.
- The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiration date indicated 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 sunlight and from heat.

4. Materials

4.1 Contents of the kit

REF	Symbol	Reagent	Content	Colour Code	
BA R-0030	PREC-REAG	**Precipitating Reagent**	2 x 55 ml	yellow	ready for use, goat anti-rabbit serum in PEG phosphate buffer *Mix thoroughly before use!*
BA R-3401	STANDARD A	**Standard A**	1 x 4 ml	white	ready for use
BA R-3402	STANDARD B	**Standard B**	1 x 4 ml	light yellow	ready for use
BA R-3403	STANDARD C	**Standard C**	1 x 4 ml	orange	ready for use
BA R-3404	STANDARD D	**Standard D**	1 x 4 ml	dark blue	ready for use
BA R-3405	STANDARD E	**Standard E**	1 x 4 ml	light grey	ready for use
BA R-3406	STANDARD F	**Standard F**	1 x 4 ml	black	ready for use
BA R-3410	AS MEL	**Melatonin Antiserum**	1 x 3 ml	blue	ready for use, from rabbit, blue coloured
BA R-3313	ASSAY-BUFF	**Assay Buffer**	1 x 15 ml	light purple	ready for use
BA R-3315	ENZYME	**Enzyme**	4 x	pink	lyophilized
BA R-3316	ENZYME-BUFF	**Enzyme Buffer**	1 x 15 ml	orange	ready for use
BA R-3320	^{125}I-MEL	**^{125}I – Melatonin**	1 x 3 ml	red	ready for use, activity < 200 kBq, red coloured
BA R-3450	ADJUST-BUFF	**Adjustment Buffer**	2 x	light purple	lyophilized
BA R-3451	CONTROL 1	**Control 1**	1 x 4 ml	light green	ready for use
BA R-3452	CONTROL 2	**Control 2**	1 x 4 ml	dark red	ready for use

4.2 Additional materials and equipment required but not provided with the kit

- Calibrated precision pipettes to dispense volumes between 15 – 1000 µl; 3 ml
- 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
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage

Saliva has to be collected in adequate sampling devices. The patient should not eat, drink or brush teeth for 30 min before sampling. Samples should be collected without stimulation and be frozen at -20 °C immediately. After thawing, the samples have to be centrifuged for 10 minutes (2,000 – 3,000 x g) immediately before the performance of the RIA. 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 500 x g 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 by vortexing and leave it 30 minutes on a rotating mixer (*please make sure that the lyophilisate is dissolved completely!*). The reconstituted enzyme cannot be stored and can only be used once. Upon request additional Enzyme vials are provided.

Adjustment Buffer
Reconstitute the content of the vial with 3 ml of water (deionized, distilled, or ultra-pure) prior to use. Mix by vortexing and leave it 60 minutes on a rotating mixer. Reconstituted Adjustment Buffer which is not used immediately has to be frozen at -20 °C (in aliquots) and may be thawed only once.

6.2 Melatonin RIA (Saliva)

1.	Pipette **15 µl** of **standards** and **controls** into the respective tubes.
2.	Pipette **500 µl** of **water** (deionized, distilled, or ultra-pure) into the tubes for **NSB, standards** and **controls.**
3.	Pipette **500 µl** of the **samples** into the respective tubes.
4.	Add **25 µl** of **Enzyme** (refer to 6.1*)* into all tubes ***(except totals)*** and vortex.
5.	Incubate for **1 h** at **RT** (20 - 25 °C).
6.	Pipette **50 µl** of **Assay Buffer** into all tubes ***(except totals)*** and mix shortly.
7.	Pipette **50 µl** of **Adjustment Buffer** into all tubes ***(except totals)*** and mix shortly.
8.	Pipette **25 µl** of the **^{125}I Melatonin** into all tubes.
9.	Pipette **25 µl** of **Melatonin Antiserum** into all tubes ***(except totals and NSB)***; mix thoroughly.
10.	Cover the tubes. Incubate for **20 - 24 h** at **RT** (20 - 25 °C).
11.	Mix the chilled (2 - 8 °C) **Precipitating Reagent** thoroughly, pipette **1000 µl** into each tube ***(except totals)***, and mix on a vortex.
12.	Incubate for **20 min** at **2 - 8 °C**.
13.	Centrifuge for **20 min** at **3 000 x g**, if possible in a refrigerated centrifuge.
14.	**Decant** or aspirate the **supernatant** **carefully** ***(except totals)***. Leave the tubes upside for 2 - 5 min.
15.	**Count** all tubes for **1 min** in a gamma counter.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Melatonin (pg/ml)	0	3	9	30	90	300
Melatonin (pmol/l)	0	12.9	39	129	390	1,290
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).

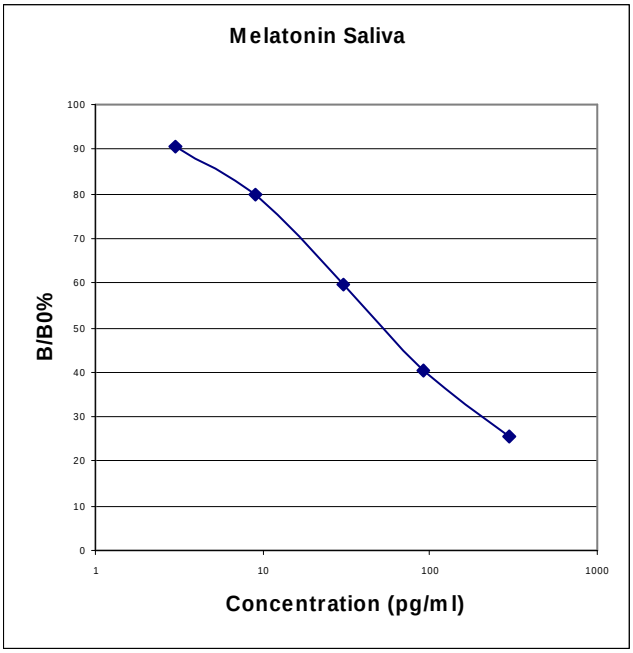
The concentrations of the **saliva samples** and the **controls** can be read directly from the Melatonin Saliva calibration curve.

7.1 Quality control

It is recommended to use control samples according to national 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 curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Expected Reference Values	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. Saliva melatonin levels in humans show a marked circadian rhythm characterized by very low levels during day time (< 5 pg/ml) and high levels during night time (> 10 pg/ml). The highest concentrations are found with infants up to the age of 3 years.

	Melatonin Saliva
Analytical Sensitivity (Limit of Detection)	1.4 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 (n = 40)				**Inter-Assay** (n = 5)			
	Sample	Range (pg/ml)	CV (%)		Sample	Range (pg/ml)	CV (%)
Melatonin Saliva	1	10.1 ± 1.0	10.3	Melatonin Saliva	1	12.9 ± 2.6	19.8
	2	31.9 ± 2.5	8.0		2	40.8 ± 4.6	11.4
	3	181.1 ± 28.6	15.8		3	224 ± 28.4	12.7

Linearity		Serial dilution up to	Range (%)	Mean (%)
	Melatonin Saliva	1 : 64	83 - 112	97

Recovery		Range (pg/ml)	Range (%)	Mean (%)
	Melatonin Saliva	9.6 - 227	93 - 111	100

⚠ ***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!