Instructions for Use

Melatonin direct Saliva ELISA

Enzyme immunoassay for the direct, quantitative determination of melatonin in human saliva.

RE54041

96

2-8°C

EU: 

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1. INTENDED USE

Enzyme immunoassay for the direct, quantitative determination of melatonin in human saliva.

2. SUMMARY AND EXPLANATION

The pineal gland ("corpus pineale") has been called a neuroendocrine transducer because of its important role in photoperiodism. The major hormone of the pineal gland is N-acetyl-5-methoxy-tryptamine or melatonin which is synthezised from the amino acid tryptophane. Melatonin has the highest levels in plasma during nighttime. Its characteristic nocturnal surge appears to encode temporal information such as length of night. Regulation of the melatonin secretion is under neural control. Sympathetic innervation seems to play a major role via its release of noradrenaline. Altered patterns and/or levels of melatonin secretion have been reported to coincide with sleep disorders, “jet lag“, depression, stress, schizophrenia, hypothalamic amenorrhea, pregnancy, anorexia nervosa, some forms of cancer, immunological disorders as well as control of sexual maturation during puberty.
Most of the circulating melatonin is metabolized in the liver to 6-hydroxymelatonin and subsequently to 6-sulfatoxymelatonin which is excreted into the urine.

3. TEST PRINCIPLE

The assay procedure follows the basic principle of competitive ELISA whereby there is competition between a biotinylated and a non-biotinylated antigen for a fixed number of antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the free biotinylated antigen is removed by a washing step and the antibody bound biotinylated antigen is determined by use of streptavidin-peroxidase as marker and TMB as substrate. Quantification of unknowns is achieved by comparing the enzymatic activity of unknowns with a response curve prepared by using known standards.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
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. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause irritations and burns.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the indicated expiry after the kit is broken. Make sure that the broken bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Saliva

The patient should not eat, drink, chew gums or brush teeth for 30 min before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination). Saliva can be collected in a suitable sampling device. A minimum of 0.5 mL liquid should be collected. Saliva flow can be stimulated by chewing on a piece of Parafilm®. It is recommended to freeze samples at –20°C prior to laboratory testing. After thawing, mix and centrifuge 10 min at 2000 – 3000 x g to remove particulate material.

Sample collection systems which contain cellulose pads should not be used.

Take care that the saliva samples are visually okay (reddish color indicates blood contamination).

Storage:	18 – 25 °C	2 – 8 °C	≤ -20 °C (Aliquots)
Stability:	24 h	1 weeks	≥ 6 months

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	**MTP**	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 10 mL 1 x 5 x 1 mL	**CAL A** **CAL B-F**	**Standard A-F** Ready to use. Contains: stabilizers. For exact concentrations see labels or QC certificate.
1 x 2 x 1 mL	**CONTROL 1+2**	**Control 1+2** Ready to use. Contains: stabilizers. For exact concentrations see labels or QC certificate.
1 x 7 mL	**ANTISERUM**	**Melatonin Antiserum** Ready to use. Contains: Antiserum (rabbit, polyclonal), stabilizers.
1 x 12 mL	**BIOTIN**	**Melatonin Biotin** Ready to use. Contains: stabilizers.
1 x 12 mL	**ENZCONJ**	**Enzyme Conjugate** Ready to use. Contains: streptavidin conjugated to HRP, stabilizers.
1 x 50 mL	**WASHBUF** **CONC**	**Wash Buffer** Concentrate (20x) Contains: phosphate buffer, Tween, stabilizers.
1 x 15 mL	**TMB SUBS**	**TMB Substrate Solution** Ready to use. Contains: TMB, Buffer, stabilizers
1 x 15 mL	**TMB STOP**	**TMB Stop Solution** Ready to use. 1 M H_2SO_4
3 x	**FOIL**	**Adhesive Foil**

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 50, 100 µL
2. A suitable sampling device should be used.
3. Orbital shaker (400-600 rpm)
4. Vortex mixer
5. 8-Channel Micropipettor with reagent reservoirs
6. Wash bottle, automated or semi-automated microtiter plate washing system
7. Centrifuge (preferably refrigerated) 2000 - 3000 x g
8. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
9. Bidistilled or deionised water
10. Paper towels, pipette tips and timer
11. Refrigerator (2-8°C)

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.
9. The relative centrifugal force (g) is not equivalent to rounds per minute (rpm) but it has to be calculated depending on the radius of the centrifuge.

10. PRE-TEST SETUP INSTRUCTIONS

	The contents of the kit for 96 determinations can be divided into 3 separate runs. **The volumes stated below are for one run with 4 strips (32 determinations).**
	**Thimerosal should be avoided in any case.**

10.1. Preparation of concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
10 mL	**WASHBUF CONC**	ad 200 mL	bidist. water	1:20	Mix vigorously.	2-8°C	4 weeks

10.2. Dilution of Samples

	**Values greater than 50 pg/mL (Standard F) must be diluted with Standard A into the linear range of the standard curve, e.g. by dilution of 1:10 (Example: 50 µL saliva + 450 µL Standard A). Dilution has to be made in glass tubes. Measured results have to be multiplied by dilution factor to obtain corrected results.** **Values lower than 0 pg/mL should be repeated by an additional measurement.**

Note: Additional Standard A with 100 mL can be ordered separately under cat. No. KESM611-100.

11. TEST PROCEDURE

1.	Pipette **100 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate.
2.	Pipette **50 µL** of **Antiserum solution** into each well. Cover plate with adhesive foil. Shake plate carefully for 10 seconds.
3.	**Incubate 16 - 20 h** at **2 - 8°C**.
4.	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.
5.	Pipette **100 µL** of **Biotin solution** into each well. Cover plate with adhesive foil.
6.	**Incubate 2 h** at **RT (18 - 25°C)** on an orbital shaker (500 rpm).
7.	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.
8.	Pipette **100 µL** of **Enzyme Conjugate** into each well. Cover plate wit adhesive foil.
9.	**Incubate 1 h** at **RT (18 - 25°C)** on an orbital shaker (500 rpm).
10.	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.
11.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
12.	**Incubate 15 min** at **RT (18 - 25°C)** on an orbital shaker (500 rpm).
13.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Shake briefly. Color changes from blue to yellow.
14.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **15 min** after pipetting of the Stop Solution.

12. 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 comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and 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.

13. CALCULATION OF RESULTS

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 Logistics 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 directly from the standard curve.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

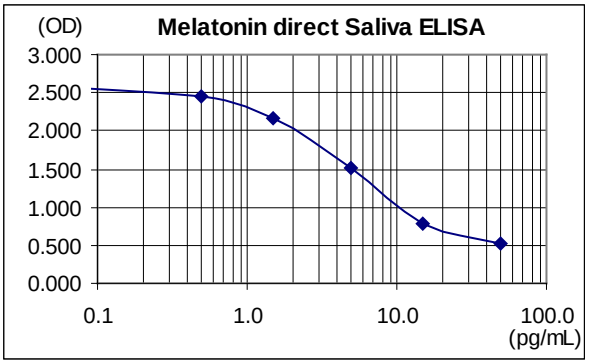
Conversion:

Melatonin (pg/mL) x 4.30 = pmol/L

Tpical Calibration Curve

(Example. Do not use for calculation!)

Standard	Concentration (pg/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0	2.666	100
B	0.5	2.464	92
C	1.5	2.176	82
D	5.0	1.506	56
E	15.0	0.794	30
F	50.0	0.519	19



14. EXPECTED VALUES

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

A study with apparently healthy subjects has shown that the melatonin levels in humans have a marked circadian rhythmicity characterised by very low levels during day-time (<5 pg/mL) and high levels during night-time (>10 pg/mL) and show a considerable inter-individual variation. Furthermore, the melatonin concentration is age dependent. The highest concentrations were found in samples of infants (up to 3 years). The nocturnal melatonin peak among healthy individuals varies significantly.

It is recommended that each laboratory establishes its own range of normal values.

15. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

Thimerosal should be avoided in any case.

The following substances do not have a significant effect on the test results up to the below stated concentrations (+/- 20%).

	Conc. in saliva
Blood	0.125 %
BSA	0.125 %
NaN_3	0.125 %

16. PERFORMANCE

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)	Cross-reactivity of other substances tested ≤ 0.01 %	
	5-Methoxytryptamine	2.5		
	N-Acetylserotonin	1.2		
	5-Methoxytryptophol	1.2		
	Serotonin	<0.02		
Analytical Sensitivity (Limit of Detection)	Saliva	0.3 pg/mL	Mean signal (Zero-Standard) - 2SD	
Functional Sensitivity	Saliva	1.0 pg/mL	Mean conc. < 20% CV	

Precision	Mean (pg/mL)	SD (pg/mL)	CV (%)	N
Intra-Assay	1.7	0.2	10.8	20
	5.1	0.3	6.1	23
	33.2	2.9	8.7	22
Inter-Assay	2.1	0.3	12.7	10
	4.9	0.4	7.6	10
	14.7	1.9	13.0	10

Linearity	Dilution	Measured (pg/mL)	Recovery (%)
	-	38.4	
	1:2	16.8	88
	1:4	8.1	84
	1:8	4.4	92
	1:16	2.5	104
	-	13.9	
	1:2	7.6	109
	1:4	4.0	115
	1:8	2.1	118
	1:16	0.9	104
	-	9.4	
	1:2	3.8	81
	1:4	2.3	98
	1:8	1.4	119
	1:16	0.7	119

Recovery	Conc. (pg/mL)	Added (pg/mL)	Measured (pg/mL)	Recovery (%)
	Saliva 1 (1.2)	0.5	1.8	106
		1.5	2.7	100
		5.0	5.5	89
		15.0	14.0	86
		30.0	27.2	87
	Saliva 2 (1.4)	0.5	2.1	111
		1.5	2.9	100
		5.0	6.5	102
		15.0	16.3	99
		30.0	30.8	98
	Saliva 3 (0.9)	0.5	1.5	109
		1.5	2.4	102
		5.0	5.1	87
		15.0	13.9	88
		30.0	27.1	88

Method Comparison versus commercial ELISA	Saliva	IBL = 0.72 x ELISA – 0.4	n = 71; r = 0.99
Method Comparison versus IBL RIA	Saliva	ELISA = 1.02 x RIA – 0.8	n = 82; r = 0.95

17. SHORT PROTOCOL

PRE TEST SET UP				
DILUTION	**Volume**	**Aqua dest.**	**Relation**	**Remarks**
WASHBUF CONC	10 mL	ad 200 mL	1:20	Example for 32 wells

SAMPLE DILUTION	Remarks
Saliva	Samples suspected to contain concentrations higher than the highest standard have to be diluted with Standard A

ASSAY PROCEDURE	
CAL A-F, CONTROL 1+2, Samples	100 µL
ANTISERUM	50 µL
Incubate **16 - 20 h at 2 - 8°C.** Aspirate the contents of each well. Wash **4 times** with **0.25 mL** of **WASHBUF (diluted)** and aspirate.	
BIOTIN	100 µL
Incubate **2 h at 18 - 25°C** on a orbital shaker (500 rpm). Aspirate the contents of each well. Wash **4 times** with **0.25 mL** of **WASHBUF (diluted)** and aspirate.	
ENZCONJ	100 µL
Incubate **1 h at 18 - 25°C** on a orbital shaker (500 rpm). Aspirate the contents of each well. Wash **4 times** with **0.25 mL** of **WASHBUF (diluted)** and aspirate.	
TMB SUBS	100 µL
Incubate **15 min. at 18 - 25°C**	
TMB STOP	100 µL
Measure optical density with a photometer at **450 nm**.	

18. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.


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