Instructions for Use

Melatonin-Sulfate Urine ELISA

Enzyme immunoassay for the quantitative determination of melatonin sulfate (synonyms: 6-hydroxymelatonin sulfate, 6-sulfatoxymelatonin) in human urine.

REF **RE54031**

Σ **96**

2-8°C

EU: IVD CE

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1. INTENDED USE

Enzyme immunoassay for the quantitative determination of Melatonin Sulfate (synonyms: 6-hydroxymelatonin sulfate, 6-sulfatoxymelatonin) in human urine.

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 its 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.
The concentration of 6-hydroxymelatonin sulfate in urine correlates well with the total level of melatonin in the blood during the collection period.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the competition principle. An unknown amount of antigen present in the sample and a fixed amount of enzyme labelled antigen compete for the binding sites of the antibodies coated onto the wells. After incubation the wells are washed to stop the competition reaction. After the substrate reaction the intensity of the developed colour is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

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 skin 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 opened. Make sure that the opened bag is tightly closed when stored at 2-8 °C.

6. SPECIMEN COLLECTION AND STORAGE

Urine

It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle. Preservation is not necessary. Determine total volume for calculation of results. **Mix and centrifuge samples before use in the assay.**

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles. For more details see: Griefahn et al. (2001).
Stability:	4 days	15 years	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 6 mL	ANTISERUM	**Melatonin Sulfate Antiserum** Ready to use. Contains: Antiserum (rabbit), Tris buffer, 0.01 % Thimerosal.
1 x 0.2 mL	ENZCONJ CONC	**Enzyme Conjugate,** Concentrate (40x) Contains: Melatonin Sulfate, conjugated to peroxidase, phosphate buffer, 0.01 % Thimerosal.
1 x 7 x 0.1 mL	CAL A-G	**Standard A-G** 0; 1.7; 5.2; 15.6; 46.7; 140; 420 ng/mL 0; 5.2; 15.9; 47.6; 142; 427; 1281 nmol/L Ready to use. Contains: Melatonin Sulfate, Tris buffer, 0.01 % Thimerosal.
1 x 2 x 0.1 mL	CONTROL 1+2	**Control 1+2** Ready to use. Contains: 0.02 % Thimerosal. Concentrations / acceptable ranges see QC certificate.
1 x 80 mL	ASSAYBUF	**Assay Buffer** Red colored. Ready to use. Contains: Tris buffer, BSA, 0.01 % Thimerosal.
1 x 50 mL	WASHBUF CONC	**Wash Buffer**, Concentrate (20x) Contains: phosphate buffer, Tween, 0.1 % Thimerosal.
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: 10; 50; 100; 1000 µL
2. Round-bottom polystyrene test tubes (12 x 75 mm)
3. Rack for test tubes
4. Orbital shaker (500 rpm)
5. Vortex mixer
6. 8-Channel Micropipettor with reagent reservoirs
7. Wash bottle, automated or semi-automated microtiter plate washing system
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

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. Some components contain ≤ 250 µL solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. 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.
8. 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.
9. 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.

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).**

10.1. Preparation of concentrated components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
15 mL	**WASHBUF CONC**	ad 300 mL	bidist. water	1:20	Resolve crystals at 18-25°C.	2-8°C	4 weeks
50 µL	**ENZCONJ CONC**	with 2 mL	ASSAYBUF	1:41	Prepare freshly and use only once.	18-25°C	30 minutes

10.2. Dilution of Standards, Controls and Patient Urine Samples

1.	Pipette **10 µL** of each **Standard, Control and patient urine sample** into polystyrene, polypropylene or glass tubes. Avoid direct sun light.
2.	Pipette **500 µL** of **Assay Buffer** into each tube. Vortex.

Samples containing concentrations higher than the highest standard have to be further diluted with Assay Buffer.

11. TEST PROCEDURE

1.	Pipette **50 µL** of each diluted **Standard,** diluted **Control and** diluted **patient sample** into the respective wells of the Microtiter Plate.
2.	Pipette **50 µL** of freshly prepared **Enzyme Conjugate** into each well.
3.	Pipette **50 µL** of **Melatonin Sulfate Antiserum** into each well.
4.	Cover plate with adhesive foil. **Incubate 2 h** at **RT (18-25°C)** on an orbital shaker (500 rpm).
5.	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.
6.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
7.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
8.	**Incubate 30 min** at **RT (18-25°C)** on an orbital shaker (500 rpm).
9.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
10.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **60 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.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

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

Calculate the 24 h excretion for each urine sample: µg/24 h = µg/L x L/24 h

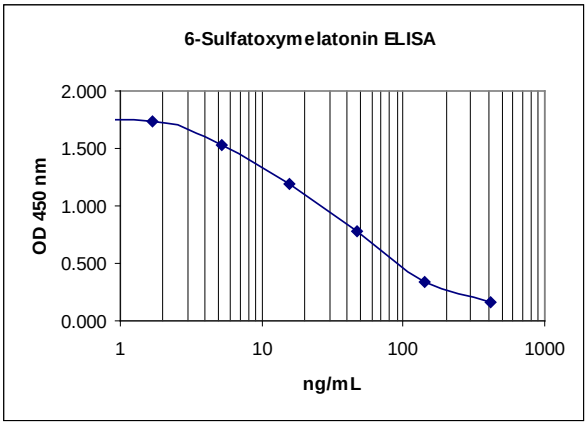
Conversion:

Melatonin Sulfate (ng/mL) x 3.05 = nmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Melatonin Sulfate (ng/mL)	OD_{Mean}	OD/OD_{max} (%)
A	0.0	1.805	100.0
B	1.7	1.741	96.5
C	5.2	1.536	85.1
D	15.6	1.185	65.7
E	46.7	0.773	42.8
F	140.0	0.341	18.9
G	420.0	0.164	9.1



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.

Apparently healthy subjects show the following values:

Age	n	Melatonin Sulfate			
		24h (µg)*		night fraction (µg/h)	
		Mean	90% percentile	Mean	90% percentile
20-35	26	36.8	15.6 - 58.1	2.8	0.9 – 5.6
36-50	17	29.6	9.9 – 52.9	2.1	0.6 – 3.6
51-65	16	20.4	12.3 – 32.8	1.5	0.9 – 2.5
> 65	16	15.8	7.5 – 32.7	1.0	0.3 – 2.3

* 24-h excretion was calculated as sum of four collection periods. For further details see: Mahlberg R. et al. Normative data on the daily profile of urinary 6-sulfatoxymelatonin in healthy subjects between the ages of 20 and 84. Psychoneuroendocrinology (2006) 31, 634-641

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.

16. PERFORMANCE

<table>
<tr><td rowspan="9">Analytical Specificity (Cross Reactivity)</td><td colspan="2">Substance</td><td>Cross Reactivity (%)</td><td colspan="2" rowspan="9">Cross-reactivity of other substances tested < 0.0001 %</td></tr>
<tr><td colspan="2">Melatonin Sulfate</td><td>100</td></tr>
<tr><td colspan="2">Melatonin</td><td>0.002</td></tr>
<tr><td colspan="2">6-OH-Melatonin</td><td>0.001</td></tr>
<tr><td colspan="2">N-Acetyl-L-OH-Tryptamine</td><td>0.0005</td></tr>
<tr><td colspan="2">N-Acetyl-L-Tryptophan</td><td>< 0.0001</td></tr>
<tr><td colspan="2">5-Methoxy-Tryptamine</td><td>< 0.0001</td></tr>
<tr><td colspan="2">Tryptamine</td><td>< 0.0001</td></tr>
<tr><td colspan="2">5-HIAA</td><td>< 0.0001</td></tr>
<tr><td>Analytical Sensitivity (Limit of Detection)</td><td>1.0 ng/mL</td><td colspan="4">Mean signal (Zero-Standard) - 2SD</td></tr>
<tr><td>Precision</td><td>Range (ng/mL)</td><td>CV (%)</td><td colspan="3" rowspan="3"></td></tr>
<tr><td>Intra-Assay</td><td>5.8 - 204</td><td>5.2 – 12.2</td></tr>
<tr><td>Inter-Assay</td><td>12.4 – 220</td><td>5.1 – 14.9</td></tr>
<tr><td rowspan="2">Linearity</td><td>Range (ng/mL)</td><td colspan="2">Serial dilution up to</td><td>Range (%)</td><td rowspan="2"></td></tr>
<tr><td>96.5 – 248.8</td><td colspan="2">1:32</td><td>80 - 116</td></tr>
<tr><td rowspan="2">Recovery</td><td>Mean (%)</td><td>Range (%)</td><td colspan="3" rowspan="2">% Recovery after spiking</td></tr>
<tr><td>105.8</td><td>91 - 122</td></tr>
<tr><td>Method Comparison versus RIA</td><td colspan="3">IBL-Assay = 1.15 x RIA + 4.2</td><td colspan="2">r = 0.96; n = 40</td></tr>
</table>

17. PRODUCT LITERATURE REFERENCES

1. Kondo, M. Tokura H., Wakamura T., Hyun KJ., Tamotsu S., Morita T., Oishi T., Influence of twilight on diurnal variation of core temperatuer, its nadir, and urinary 6-hydroxymelatonin sulfate during nocturnal sleep and morning drowsiness. Coll. Antropol. 33 (2009) 1: 193-199
2. Radwan P., Skrzydlo-Radomanska B., Radwan-Kwiatek K., Burak-Czapiuk B., Strzemecka J. Is melatonin involved in the irritable bowel syndrome? Journal of Physiology and Pharmacology (2009), Suppl 3, 67-70
3. Mahlberg R. et al. Normative data on the daily profile of urinary 6-sulfatoxymelatonin in healthy subjects between the ages of 20 and 84. Psychoneuroendocrinology (2006) 31, 634-641
4. Kunz et al. Melatonin in Patients with Reduced REM Sleep Duration: Two Randomized Controlled Trials. Journal of Clinical Endocrinology & Metabolism, 89(1): 128-134 (2004)
5. Mahlberg et al. Melatonin and age. Somnologie 7 (Suppl. 1): 9-88 (2003)
6. Griefahn et al. Melatonin production during childhood and adolescence: a longitudinal study on the excretion of urinary 6-hydroxymelatonin sulfate. J. Pineal Res. 34:26-31 (2003)
7. Bortkiewicz et al. The excretion of 6-hydroxymelatonin sulfate in healthy young men exposed to electromagnetic fields emitted by cellular phone – an experimental study. Neuroendocrinology Letters 23(suppl 1):88-91 (2002)
8. Kunz et al. Exogenous Melatonin in Periodic Limb Movement Disorder: An Open Trial and a Hypothesis. SLEEP, Vol. 24, No. 2, 183-187, (2001).
9. Griefahn et al. Long-Term Stability of 6-Hydroxymelatonin Sulfate in 24-h Urine Samples Stored at –20°C. Endocrine, vol. 15, no. 2, 199-202, July (2001).
10. Kunz D et al. Melatonin as a therapy in REM sleep behavior disorder patients: an open-labeled pilot study on the possible influence of melatonin on REM-sleep regulation. Movement Disorders, 14: 507-511 (1999)
11. Pfluger DH, Minder CE. Effects of exposure to 16.7 Hz magnetic fields on urinary 6-hydroxymelatonin sulfate excretion of Swiss railway workers. J. Pineal Res., 21: 91-100 (1996)

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.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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