DRG® Melatonin-Sulfate (EIA-1432)

USA: RUO

Revised 12 Sept. 2011 rm (Vers. 8.1)

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit

INTENDED USE

Enzyme immunoassay for determination of melatonin sulfate (synonyms: 6-Hydroxymelatonin Sulfate, 6-Sulfatoxymelatonin) in human urine.

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 color is inversely proportional to the amount of the antigen in the sample. Results of samples can be determined directly using the standard curve.

WARNINGS AND PRECAUTIONS

1. For research 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 DRG or your supplier in written form, latest one week after receiving the kit. Don't use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Don't mix reagents of different lots. Don't 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 upon request.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.
The microtiter plate is stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

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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 sun light. Avoid repeated freeze-thaw cycles. For more details see: Griefahn et al. (2001).
Stability:	4 d	15 y	

MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12x8	MTP	**Microtiter Plate,** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 5 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**; Ready to use. 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 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 60 mL	ASSAYBUF	**Assay Buffer,** Ready to use. Red colored. 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 12 mL	TMB SUBS	**TMB Substrate Solution,** Ready to use. Contains TMB, buffer, stabilizers.
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1 M H_2SO_4.
3 x	FOIL	**Adhesive Foil**

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 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

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. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate 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.

PRE-TEST SETUP INSTRUCTIONS

Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component		Diluent	Rela-tion	Remarks	Storage	Stability
15 mL	**Wash Buffer**	ad 300 mL	bidist. water	1:20	Resolve crystals at 18 – 25 °C.	2 – 8°C	4 w
50 µL	**Enzyme Conjugate**	with 2 mL	Assay Buffer	1:41	Prepare freshly and use only once.	18 – 25°C	30 min

Dilution of Standards, Controls and Urine Samples

1. Pipette **10 µL** of each **Standard, Control and 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.

TEST PROCEDURE

1. Pipette **50 µL** of each diluted **Standard,** diluted **Control and** diluted **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.

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 other applicable standards/laws. All kit controls must be found

within the acceptable ranges as stated at 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.

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 Logisitcs 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 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/24h = µg/L x L/24h

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

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Melatonin Sulfate (ng/mL)	Mean OD	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	0.341	18.9
G	420	0.164	9.1

PRODUCT LITERATURE REFERENCES

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