DRG® Melatonin Saliva (Non-extraction) (SLV-4779)

Revised 2 Feb. 2011 rm (Vers. 3.1) **USA: RUO**

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

This kit is intended for Research Use Only.

This kit is not intended for diagnostic purposes.

1 INTENDED USE

Enzyme immunoassay for determination of melatonin in human saliva.

2 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.

3 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. 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 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 irritations and burns.

4 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 strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2 - 8°C.

Revised 2 Feb. 2011 rm (Vers. 3.1) **USA: RUO**

5 SPECIMEN COLLECTION AND STORAGE

Saliva

The sample subject 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.

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°C - 25°C	2°C - 8°C	≤ -20°C (Aliquots)
Stability:	24 h	1 w	≥ 6 mon

6 MATERIALS SUPPLIED

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

7 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 (can be ordered separately from DRG under REF SLV-4158)
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)

8 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. 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.
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.

9 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.**

9.1 Preparation of lyophilized or concentrated components

Dilute/ dissolve	Component		Diluent	Rela-tion	Remarks	Storage	Stability
10 mL	WASHBUF CONC	ad 200 mL	bidist. water	1:21	Mix vigorously.	2-8°C	4 w

9.2 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.

10 TEST PROCEDURE

1. Pipette **100 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate. Use **100 µL Standard A** for Blank wells.
2. Pipette **50 µL** of **Antiserum solution** into each well, except Blank wells. 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 with 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.

11 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 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:

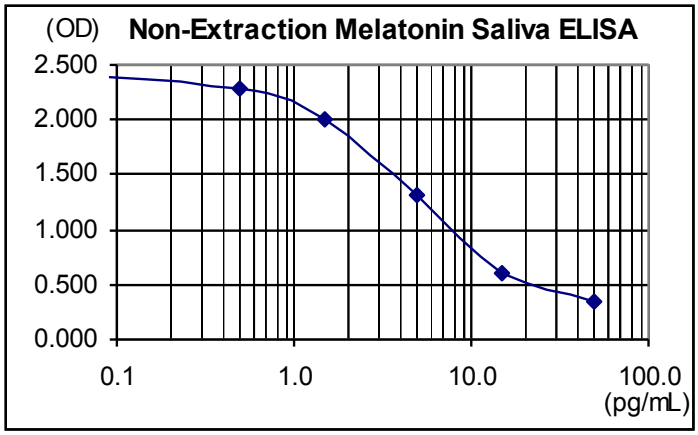
Melatonin (pg/mL) x 4.30 = pmol/L

Typical Calibration Curve

(Example. Do not use for calculation!)

Revised 2 Feb. 2011 rm (Vers. 3.1) **USA: RUO**

Standard	Concentration (pg/mL)	OD - Blank	B/Bmax (%)
A	0.0	2,485	100
B	0.5	2,283	92
C	1.5	1,995	80
D	5.0	1,325	53
E	15.0	0,613	25
F	50.0	0,338	14
Blank	-	0,181	7



12 SHORT PROTOCOL

PRE TEST SET UP				
DILUTION	**Volume**	**Aqua dest.**	**Relation**	**Remarks**
WASHBUF CONC	10 mL	200 mL	1:21	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, Blank	100 µL
ANTISERUM	50 µL (except Blank)
Incubate **16 - 20 h at 2 - 8°C.** Aspirate the contents of each well. Wash **4 times** with **0.25 mL** of **diluted Wash Buffer** and aspirate.	
BIOTIN	100 µL
Incubate **2 h at 18 - 25°C** on a orbital shaker (500rpm). Aspirate the contents of each well. Wash **4 times** with **0.25 mL** of **diluted Wash Buffer** and aspirate.	
ENZCONJ	100 µL
Incubate **1 h at 18 - 25°C** on a orbital shaker (500rpm). Aspirate the contents of each well. Wash **4 times** with **0.25 mL** of **diluted Wash Buffer** 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**.	

13 REFERENCES

1. Iriti, M., Rossoni, M, Faoro F. Melatonin content in grape myth or panacea? J Sci Food Agric 86:1432-1438 (2006)
2. Lahiri, D. K., Ge, Y.-W., Sharman, E. H., Bondy, St. C., Age-related changes in serum melatonin in mice: higher levels of combined melatonin and 6-hydroxymelatonin sulfate in the cerebral cortex than serum, heart, liver and kidney tissues. J. Pineal Res. May 2004, Vol. 36, issue 4, 217-223
3. Sharman E et al. Age-related changes in murine CNS mRNA gene expression are modulated by dietary melatonin. J. Pineal Res. Vol 36, Issue 3: 165ff. (2004)
4. Wagner H.-J. Mattheus U. Pineal organs in deep demersal fish. Cell Tissue Res 307:115-127 (2002)
5. 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)
6. 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)
7. Follenius M, Weibel L, Brandenberger G. Distinct modes of melatonin secretion in normal men. J. Pineal Res., 18: 135-140 (1995)
8. Dubbels R et al. Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. J. Pineal Res., 18. 28-31 (1995)
9. Czeisler CA et al. Suppression of melatonin secretion in some blind patients by exposure to bright light. N. Engl. J. Med., 332: 6-11 (1995)