Direct Saliva MELATONIN ELISA

EK-DSM 96 tests

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INTENDED USE

The NovoLytiX Direct Saliva Melatonin ELISA (EK-DSM) is intended for highly sensitive, quantitative determination of melatonin in human saliva (1-4).

PRINCIPLE OF THE ASSAY

The NovoLytiX Direct Saliva Melatonin ELISA is a competitive immunoassay using a capture antibody (Ab) technique. The polyclonal Kennaway G280 anti-melatonin antibody (5, 6) has been coated onto the microtiter plate, provided in the kit. During the 16-20-hours overnight incubation melatonin present in the pre-treated saliva and controls as well as in the calibrators compete with biotinylated melatonin for the binding sites of this highly specific antibody. After washing, the enzyme label, streptavidin conjugated to horseradish peroxidase (HRP), is added, which binds to the melatonin-biotin-antibody complexes captured on the coated wells during a 60-minutes incubation step. Unbound enzyme label is then removed by a second washing step and TMB substrate (tetramethylbenzidine) is added to the wells. In a further 30-minutes incubation step, a chromophore is formed in inverse proportion to the amount of melatonin present in the sample. The color turns from blue to yellow after the addition of an acidic stop solution and can be measured at 450 nm.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Pretreatment Solution	1 vial 5 mL	B-PRS	Ready to use Corrosive agent
Neutralizing Solution	1 vial 5 mL	B-NS	Ready to use
Microtiter Plate precoated with G280 anti-melatonin Ab	12x8 wells	B-EKDSM-MP	Wash 2x before use
Plate Sealer	3 pieces	-	Ready to use
Wash Buffer Concentrate (10x) with preservatives	1 bottle 100 mL	B-WB1	Dilute with 900 mL deionized water
Blanking Reagent[1)]	1 vial 1 mL	B-EKDSM-BR	Ready to use
Zero Calibrator melatonin-free buffer	1 vial 6 mL	B-EKDSM-0CAL	Ready to use
Calibrators[2)]	5 vials 1 mL	B-EKDSM-CASET	Ready to use
Control low / high[3)] Ready for pretreatment (see page 3)	2 vials 1 mL	B-EKDSM-CONSET	Ready to use
Biotin Conjugate	1 vial 3 mL	B-EKDSM-BC	Ready to use
Enzyme Label Streptavidin conjugated to HRP	1 vial 11 mL	B-EKDSM-EL	Ready to use
TMB Substrate buffered with citrate	1 vial 11 mL	B-TMB	Ready to use
Stop Solution	1 vial	B-STS	Ready to use
0.25 M sulfuric acid (H_2SO_4)	11 mL		Irritant

Table 1

[1)] The Blanking reagent contains a saturated melatonin solution. Prevent any contamination of other kit reagents.
[2)] The Calibrators A, B, C, D and E contain the following melatonin concentrations: 0.4, 1.2, 4, 12 and 40 pg/mL which are corrected for the 20% sample dilution during pretreatment and therefore labeled with 0.5, 1.5, 5.0, 15, and 50 pg/mL of melatonin, respectively.
[3)] Lot specific amount of melatonin, see QC data sheet added to the kit.

STORAGE AND SHELF LIFE OF REAGENTS

Sealed / Unopened Reagents	
Store at 2-8°C until expiration date. Do not use past expiration date.	
Opened / Reconstituted Reagents	
Microtiter Plate	Return unused strips immediately to the aluminium pouch containing the desiccant pack and reseal along the entire edge of zip-seal. Store for up to 2 months at 2-8°C.
Pretreatment Solution	Store at 2-8 °C until expiration date printed on the labels.
Neutralizing Solution	
Incubation Buffer	
Blanking Reagent	
Calibrators	
Controls	
Biotin Conjugate	
Enzyme Label	
TMB Substrate	
Stop Solution	
Wash Buffer diluted	Store at 2-8°C up to 6 months

Table 2

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes with disposable tips: 5 µL, 25 µL, 100 µL and 1000 µL pipettes. Repeater or multichannel pipette for 25 µL and 100 µL.
- Disposable polystyrene or polypropylene tubes for the preparation of sample pretreatment/neutralization and dilutions.
- 1000 mL cylinder for the dilution of the wash buffer.
- Eppendorf or alternative microfuge able to hold a speed of at least 10’000 rpm.
- Microtiter plate washer or squeeze bottle for wash buffer.
- Blotting paper.
- Refrigerator.
- Microtiter plate orbital shaker.
- Microtiter plate reader for measurement of absorbance at 450 nm.
- NovoLytiX Saliva Collection Devices, B-SVC50-U or B-SLEEPCHECK16 (optional).

PRECAUTIONS

Safety precautions

- This test must be handled by qualified personnel, in accordance with good laboratory practices (GLP).
- The pretreatment solution (B-PRS) contains sodium hydroxide (NaOH). The stop solution (B-STS)

contains sulfuric acid (0.25 M). Each of those reagents is irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothes Wear suitable protective clothing, gloves and eye protection. After contact with eyes or skin, wash immediately with plenty of water.

Unused solutions should be disposed of according to local, state and federal regulations.

Technical precautions

Kit components

- Read the instructions carefully before carrying out the test. Test performance will be adversely affected if reagents are incorrectly diluted, modified or stored under conditions other than those as detailed in this instruction for use.
- Residues in the microtiter plate wells result from the production process. They are removed in the washing step (assay procedure step 2) and do not affect the results.
- Components must not be used beyond the expiry date printed on the labels.
- Do not mix different lots of reagents.
- Every effort should be made to ensure that no cross contamination occurs between reagents, samples or between wells.
- Microwells cannot be re-used.
- Let the reagents adjust to reach room temperature and mix (vortex) the reagents well before use.

Assay procedure

- The blanking reagent contains a saturated melatonin solution. Avoid any contamination of other reagents of this kit. Change disposable tips after each pipetting step.
- Blank reagent, calibrators and controls must be assayed in duplicates. Duplicates for patient samples are also strongly recommended, but many users prefer single determinations. This approach allows to test up to 39 samples in duplicates or 78 samples as singles per microtiter plate.

SPECIMEN STORAGE AND SHIPMENT

Storage: The saliva samples absorbed in the cotton swab may be stored in the saliva collection device for up to 7 days at 2-8°C. If not assayed within one week after collection, samples should be frozen and may be stored for at least 12 months at ≤ -20°C. Repeated freeze-thaw cycles should be avoided.

Shipment: Collected saliva samples must be kept in the refrigerator at 2-8°C for up to seven days or at ≤ -20°C before shipment. The collected saliva samples can be shipped to the testing laboratory at ambient temperatures if the shipment does not take longer than 72 hours. However, the shipment on blue-ice or on dry ice is highly recommended.

Samples should not be sent on Fridays, Saturdays or the day before holiday to prevent unnecessarily extended shipping times.

SPECIMEN COLLECTION

Collect saliva using the NovoLytiX Saliva Collection Devices (order code: B-SVC50-U). The devices can absorb up to 3 mL of saliva. The procedure calls for at least 0.2 mL of saliva. Alternative sampling devices and procedures, respectively, may also work well, but:

- Do not use cotton swabs containing citric acid.
- Do not stimulate saliva flow by chewing gums or eating lemons.
- Patients should perform the collection on an evening without sporting activities and any intense efforts.
- When collecting saliva at night, a dim flash light or a ≤100 lux yellow light should be used in order to avoid a possible light influence (suppression) on the melatonin profile.
- Nothing should be eaten during the collection time. The last meal must be taken at least 30 minutes before starting the collection. Bananas and chocolate should not be eaten during the entire day before the collection. Rinse the mouth with a fewe mL of water 5 minutes before the collection.
- Drinks containing artificial colorants, caffeine (coffee, black or green tea, iced tea, cola) or alcohol are to be avoided during the entire collection period.
- Patients should avoid brushing their teeth, with or without toothpaste, during sampling periods, but at least 30 minutes before next sampling. If toothpaste is used nevertheless, rinse the mouth with plenty of water. It is likely that patients with gingivitis will contaminate the saliva with blood leading to unknown consequences.
- On the collection day, if possible, no aspirin and medicines that contain ibuprofen (Brufen®, Algifor® Dismenol®, Dolocyl®, Ecoprofen®) should be taken. Norepinephrine (noradrenaline) stimulates melatonin synthesis and treatment shall be stopped at least 36 hours before saliva sampling. If a subject is treated with melatonin, melatonin intake must be discontinued at least three days before the collection period.

SAMPLE PRETREATMENT (LABORATORY)

Sample recovery from saliva collection devices

Centrifuge the collection devices sent by the patient for 5 min at 3000 rpm (1500x g). Discard the suspended insert with the swab and store the tube at 2-8°C (for up to 7 days)or at -20°C (for up to 12 months).

Pre-treatment of saliva samples and controls

1. Pipet 200 µL of controls and saliva samples, respectively, into correspondingly marked polypropylene tubes.
2. Add 25 µL of pretreatment solution to each tube using a multichannel or repeater pipette.
3. Vortex for 5 seconds and leave the tubes for 10 minutes at 18-28 °C.
4. Add 25 µL of neutralizing solution to each tube using a multichannel or repeater pipette. Vortex for 5 seconds.
5. Centrifuge the pre-treated samples for 5 minutes at 10’000 rpm. Proceed to the ELISA assay procedure.

ASSAY PROCEDURE

1. Use a plate with enough 8-well strips to test the desired number of blanks, calibrators, controls and samples. Remove excess strips from the holder and re-seal them in the foil pouch together with the two desiccant bags **without delay**. Store refrigerated.
2. Wash the coated strips twice using at least 300 µL of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

3a. Pipet 100 µL of Blanking Reagent (blank) in duplicate into wells A1+A2.

3b. Pipet 100 µL of Zero Calibrator in duplicate into wells B1+B2.

3c. Pipet 100 µL of Calibrator A in duplicate into wells C1+C2.

Pipet 100 µL of Calibrator B in duplicate into wells D1+D2.

Pipet 100 µL of Calibrator C in duplicate into wells E1+E2.

Pipet 100 µL of Calibrator D in duplicate into wells F1+F2

Pipet 100 µL of Calibrator E in duplicate into wells G1+G2

3d. Pipet 100 µL of pretreated low control in duplicate into wells H1+H2

Pipet 100 µL of pretreated high control in duplicate into wells A3+A4

3e. Pipet 100 µL of each pretreated sample (singles or duplicates) into the subsequent wells.

4. Add 25 µL of Biotin Conjugate (blue solution) to each well. Cover the plate with a plate sealer and place it for 1 min on a plate orbital shaker set at 600 rpm.
5. Incubate for 18±2 hours at 2-8 °C.
6. Remove and discard the plate sealer. Aspirate or invert the plate to empty the solution from each well and wash five times using at least 300 µL of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
7. Add 100 µL of Enzyme Label (yellow solution) to all wells.
8. Cover the plate with a new plate sealer, place the plate on a plate orbital shaker set at 600 rpm and incubate for 60 minutes (±5 minutes) at 18-28 °C.

Important: *allow the TMB substrate to equilibrate to 18-28°C prior to use in step 10.*

9. Remove and discard the plate sealer. Aspirate or invert the plate to empty the solution from each well and wash five times using at least 300 µL of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
10. Add 100 µL of TMB substrate to all wells.
11. Cover the plate, place it on a plate orbital shaker set at 600 rpm, protect the plate from direct light and incubate for 30±5 minutes at 18-28°C.
12. Add 100 µL of Stop Solution to all wells. Remove air bubbles by pricking them with a pipette tip. Proceed to step 13 within 30 minutes.
13. Read the absorbance at 450 nm in a microtiter plate reader.

QUALITY CONTROL

A thorough understanding of this instruction for use is necessary for the successful use of the product. Reliable results will be obtained only by precise laboratory techniques (current GLP guidelines) and accurately following this instruction for use.

Since there are no controls for salivary melatonin commercially available, we recommend using saliva pools containing different levels of melatonin for internal quality control.

The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. The confidence limits for the controls are lot-specific and printed on the additional QC data sheet.

If the performance of the assay does not meet the established limits and repetition has excluded errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices, ii) ELISA reader settings, iii) expiration dates of reagents, iv) storage and incubation conditions, v) TMB substrate solution should be colorless, and vi) purity of water.

STANDARDIZATION

The NovoLytiX Direct Saliva Melatonin ELISA is calibrated with United States Pharmacopeia (USP) Reference Standard material (Merck #1380105), and its correct concentration used to generate the kit Calibrators was confirmed by UV/VIS: ε_{278} = 6300 $M^{-1}cm^{-1}$ in ethanol/H_2O solution.

RESULTS

Standard Curve

Record the absorbance at 450 nm for each calibrator and blank well. Average the duplicate values, subtract the average of the blank wells and record averages (=corrected average absorbance). Calculate the binding (B) of each pair of calibrator wells as a percent of Zero Calibrator (B_0), with the blank-corrected absorbance of the Zero Calibrator taken as 100 %.

$$B/B_0\ (\%) = \text{percent bound} = \frac{\text{net absorbance}}{\text{net absorbance of Zero Calibrator}} \times 100$$

Plot the percent bound (vertical axis) versus the concentration of melatonin in pg/mL (horizontal axis) using a lin/log graph paper. Draw the best fitting curve or calculate the standard curve using a four-parameter logistic (4-PL) or any other suitable algorithm.

Samples and controls

- Record the absorbance at 450 nm for each sample, each control well(s). Subtract the average of the blank wells and record the absorbance (=corrected average absorbance). Calculate, as described above, the binding of each pair of sample wells as a percent of Zero Calibrator (B_0), with the blank-corrected absorbance of Zero Calibrator taken as 100%.
- Locate the B/B_0 value of the samples on the vertical axis, draw a horizontal line intersecting the standard curve and read the melatonin concentration (pg/mL) from the horizontal axis.

See table 3 and figure 1 for examples of results and standard curve. *The presented results and standard curve are for demonstration purposes only. A fresh standard curve must be generated for each set of samples to be assayed.*

LIMITATIONS

Melatonin results should be interpreted in conjunction with information available from the study set-up, (clinical) assessment of the subject and other interventional procedures.

PERFORMANCE CHARACTERISTICS

Repeatability: 4.9 – 17.7% CV
Within-laboratory precision: 9.7 – 19.8% CV
Repeatability, between-run, between-day and within-laboratory (inter-assay) precision were established based on the CLSI guideline EP05-A2 using a 15 days x 2 runs x 2 replicates study design. Ten saliva samples with melatonin concentrations ranging from 1.4 – 49.3 pg/mL were tested (table 4).

Limit of Detection (LoD): 0.47 pg/mL
The LoD was established according to the CLSI guideline EP17-A and with proportions of false positives (α) less than 5% and false negatives (β) less than 5% based on 160 determinations, with 40 blank (Zero Calibrator) and 120 low level replicates; and a **Limit of Blank (LoB) of 0.22 pg/mL**.

Limit of Quantitation (LoQ): 1.3 pg/mL
The LoQ was established using data obtained in the within-laboratory precision study, including an additional saliva sample with a concentration of 1.1 pg/mL. The LoQ was determined as the melatonin concentration at which the non-linear fit of total precision data intersected the precision goal of 20% CV.

Linearity: 0.6 – 50 pg/mL

The linear range of the NovoLytiX Direct Saliva Melatonin ELISA was determined according to the CLSI guideline EP06-A. A maximum deviation from linearity of ±20% was allowed (table 5).

Spiking Recovery: 99.3%. Two saliva sample pools containing 1.4 and 1.7 pg/mL endogenous melatonin, respectively, were spiked with 1 to 31 pg/mL of exogenous melatonin and assayed according to the assay procedure. The results are presented in table 6.

Specificity: The 50% binding (cross-reactivity) of the melatonin antiserum with different compounds was tested in the NovoLytiX Direct Saliva Melatonin Radioimmunoassay (RK-DSM2) and are presented in table 7. The data can be transferred to the NovoLytiX Direct Saliva Melatonin ELISA as the same antibody (G280) in very similar assay buffer systems as compared to the RK-DSM2 assay are used.

Method Comparison: The comparison was done with 119 saliva samples from 20 different donors collected at different daytimes. The samples were analyzed using the presented NovoLytiX Direct Saliva Melatonin ELISA (EK-DSM) as well as the NovoLytiX Direct Saliva Melatonin Radioimmunoassay (RK-DSM2). The Passing-Bablok scatter plot shows an almost identical performance of the two immunoassays (y-intercept = +0.01 pg/mL; slope = 0.99). The Spearman's correlation factor, rs, is 0.967. The comparison data are presented in figure 2.

APPENDIX I

TABLES AND FIGURES

Examples of Results

	Conc. (pg/mL)	Absorbance (OD)	B/B0 (%)	CV Conc. (%)	Calc. Conc. (pg/mL)
Blank Blank **Avg.**		0.092 0.084 **0.088**			
Zero Calibrator Zero Calibrator **Avg.**	**0.0**	1.974 1.986 **1.980**	99.7 100.3 **100.0**	**0.3**	
Cal A Cal A **Avg.**	**0.5**	1.824 1.817 **1.821**	92.1 91.8 **91.9**	**0.3**	
Cal B Cal B **Avg.**	**1.5**	1.600 1.590 **1.595**	80.8 80.3 **80.6**	**0.4**	
Cal C Cal C **Avg.**	**5**	0.980 0.991 **0.986**	49.5 50.1 **49.8**	**0.8**	
Cal D Cal D **Avg.**	**15**	0.477 0.490 **0.483**	24.1 24.7 **24.4**	**1.9**	
Cal E Cal E **Avg.**	**50**	0.200 0.225 **0.212**	10.1 11.4 **10.7**	**8.3**	
Ctrl. high Ctrl. high **Avg.**		0.483 0.527 **0.505**	24.4 26.6 **25.5**	**8.5**	14.9 13.2 **14.1**
Ctrl. low Ctrl. low **Avg.**		1.382 1.369 **1.375**	69.8 69.1 **69.5**	**1.9**	2.4 2.4 **2.4**
Sample 01 Sample 01 **Avg.**		1.755 1.717 **1.736**	88.6 86.7 **87.7**	**11.1**	0.8 1.0 **0.9**
Sample 02 Sample 02 **Avg.**		1.122 1.074 **1.098**	56.7 54.2 **55.0**	**6.3**	4.0 4.3 **4.1**

Table 3

ED20 = 19.6 pg/mL ED50 = 5.0 pg/mL ED80 = 1.5 pg/mL

Example of Standard Curve (OD_{450})

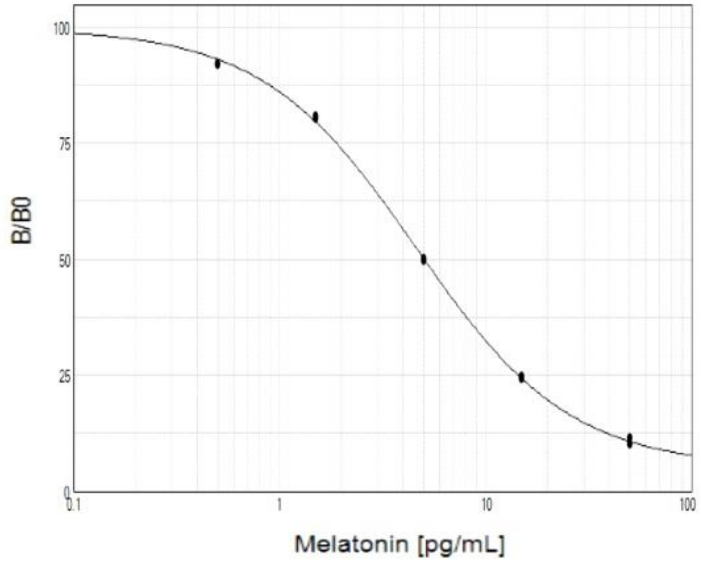

Figure 1

Precision

ID	Mean [pg/mL]	n	Repeata-bility		Between-run		Between-day		Within-laboratory	
			SD	CV	SD	%CV	SD	%CV	SD	CV
Pool1.3	1.38	60	0.09	6.8%	0.13	9.7%	0.22	15.9%	0.27	19.8%
NSC002	2.53	60	0.19	7.4%	0.18	7.1%	0.32	12.6%	0.41	16.3%
NSC003	4.45	60	0.22	4.9%	0.19	4.2%	0.38	8.5%	0.47	10.6%
Pool7	7.72	60	0.46	5.9%	0.32	4.1%	1.03	13.4%	1.18	15.2%
Pool10	8.95	59	0.54	6.0%	0.58	6.5%	0.89	9.9%	1.19	13.3%
NSC004	16.4	60	1.16	7.1%	1.10	6.7%	0.85	5.2%	1.81	11.1%
Pool20	22.4	60	2.31	10.3%	1.81	8.1%	1.86	8.3%	3.48	15.5%
Pool30	32.6	60	4.82	14.8%	0.00	0.0%	2.26	6.9%	5.33	16.3%
Pool40	40.1	60	2.77	6.9%	2.08	5.2%	1.73	4.3%	3.87	9.7%
Pool50	49.3	60	8.75	17.7%	0.00	0.0%	2.65	5.4%	9.14	18.5%

Table 4

Linearity

ID	Measuring range tested [pg/mL]	R^2	p-value for non-linear coefficient	Linear range [pg/mL]
HP1	0.50 - 63.9	0.999	p >0.05	0.51 - 65.0
HP2	0.57 - 60.5	0.988	p >0.05	0.55 - 58.4

Table 5

Spiking Recovery

ID	Spiked with [pg/mL]	Expected [pg/mL]	Observed [pg/mL]	Recovery O/E [%]
SR1	-	-	1.4	-
	1	2.4	2.5	104.2
	3	4.4	5.1	115.9
	7	8.4	8.3	98.8
	15	16.4	17.2	104.9
	31	32.4	35.7	110.2
SR2	-	-	1.7	-
	1	2.7	2.5	92.6
	3	4.7	3.8	80.9
	7	8.7	8.4	96.6
	15	16.7	15.1	90.4
	31	32.7	32.1	98.2
Mean				**99.3**

Table 6

Specificity

Compound	Crossreactivity [%]
melatonin	100
serotonin	< 0.001
6-sulfatoxymelatonin	< 0.001
N-acetylserotonin	0.045
5-hydroxy-indole acetic acid	< 0.001
5-methoxytryptamine	0.007
5-methoxytryptophane	< 0.001
2-methyl-5-hydroxytryptamine	< 0.001
5-methoxypsoralen	< 0.001
5-methoxytryptophol	0.002
6-chloromelatonin	1.3
caffeine	< 0.001
caffeic acid	< 0.001
soluble coffee	< 0.001
soluble coffee decaffeinated	< 0.001

Table 7

Comparison EK-DSM vs. RK-DSM2

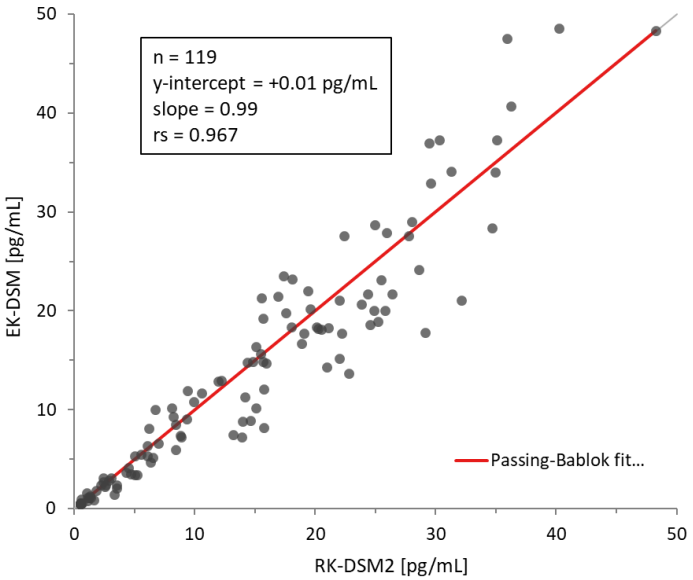

Figure 2

APPENDIX II

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APPENDIX III

CHANGE LOG

Date	Version	Reason for change
2021-05-07	01	First version after transfer from BÜHLMANN Laboratories AG to NovoLytiX GmbH
2023-05-20	02	Change of reagent codes from B-EKDSM-PRS to B-PRS, from B-EKDSM-NS to B-NS, and from B-EKDSM-WB to B-WB1, resp., as the same reagents are used for different assay kits; down rating of B-NS from irritant to not dangerous state and of B-STS from corrosive agent to irritant according to the latest REACH guidelines
2023-04-12	03	Total revision of the assay procedure and corresponding assay protocol (IFU); all calibrators and controls new ready to use; recalibration of the assay using United States Pharmacopeia (USP) Reference Standard material (Merck #1380105); extension of the calibration curve new from 0.5 to 50 pg/mL; reduction of the pipetting volume of Biotin-Conjugate (B-EKDSM-BC) from 50 µL to 25 µL; incubation with B-EKDSM-BC new overnight instead of 3 hours to stabilize the assay kinetics, therefore one incubation step less; Incubation Buffer (B-EKDSM-IB) is replaced by the Zero Calibrator (B-EKDSM-0CAL); New and updated performance characteristics, respectively, for precision, LoB, LoD, LoQ, linearity, spiking recovery, and method comparison; Norepinephrine (noradrenaline) stimulates melatonin synthesis and respective treatment shall be stopped at least 36 hours before saliva sampling (see chapter “Specimen Collection”)

APPENDIX IV

PIPETTING PROTOCOL

Direct Saliva Melatonin
Sample Pretreatment (Saliva & Controls)

Clean polypropylene tube

200 µl Saliva Sample or Control
25 µl Pretreatment Solution

Vortex, 5 sec

Incubate, 10 min, 18-28°C

25 µl Neutralization Solution

Vortex, 5 sec

Centrifuge at 10'000 rpm for 5 min

Proceed to ELISA procedure

Direct Saliva Melatonin
ELISA Procedure

Precoated Microtiter Plate

Wash 2x with ≥300µL wash buffer

100 µl Calibrators, Pretreated Controls or Samples

add 25 µl Melatonin-Biotin-Conjugate

1 minute on a plate orbital shaker

18 hours at 2-8°C

Wash 5x with ≥300µL wash buffer

add 100 µl Enzyme Label

60 minutes at 18-28°C on a plate orbital shaker

Wash 5x with ≥300µL wash buffer

add 100 µl TMB Substrate

Incubate 30 minutes at 18-28°C on a plate orbital shaker

add 100 µl Stop Solution

➡ Read absorbance at 450 nm (within 30 minutes)

NOTES

APPENDIX V

SYMBOLS

Symbol	Explanation
	Use By Verwendbar bis Utiliser jusqu'au Utilizzare entro Fecha de caducidad
REF	Order Code Bestellnummer Code Codice Código
LOT	Batch Code Lotbezeichnung Code du lot Codice del lotto Codigo de lote
IVD	*In Vitro* Diagnostic Medical Device *In Vitro* Diagnostikum Dispositif médical de diagnostic *in vitro* Dispositivo medico-diagnostico *in vitro* Producto sanitario para diagnóstico *in vitro*
	Contains sufficient for <n> tests Ausreichend für "n" Ansätze Contenu suffisant pour „n" tests Contenuto sufficiente per „n" saggi Contenudo suficiente para <n> ensayos
	Consult Instructions for Use- Gebrauchsanweisung beachten Consulter le mode d'emploi Consultare le istruzioni per l'uso Consulte las instrucciones de uso
	Temperature Limitation Zulässiger Temperaturbereich Limites de température Limiti di temperatura Limite de temperatura
SOLN PRE	Pretreatment Solution Vorbehandlungslösung Solution de prétraitement Soluzione di pretrattamento Solución del tratamiento previo
SOLN NEUT	Neutralizing Solution Neutralisierungs-Lösung Solution neutralisante Soluzione neutralizzante Solución neutraliza
MP	Microtiter plate Mikrotiterplatte Microplaque Micropiastra Placa de microtitulación

Symbol	Explanation
BUF WASH 10X	Wash Buffer Concentrate (10x) Wasch-Puffer Konzentrat (10x) Concentré de tampon de lavage (10x) Tampone di lavaggio concentrato (10x) Tampón de lavado concentrado (x10)
REAG BLANK	Blanking Reagent Nullwert-Reagenz Réactif blanc Reagente bianco Reactivo blanco
CAL 0	Zero Calibrator Null-Standard Calibrateur de zéro Calibratore di zero Calibrador cero
CAL A - CAL E	Calibrator A -E Kalibrator A -E Calibrateur A -E Calibratore A - E Calibrador A - E
CONTROL L	Control Low Kontrolle tief Contrôle bas Controllo basso Control bajo
CONTROL H	Control High Kontrolle hoch Contrôle élevé Controllo alto Control alto
BC	Biotin Conjugate Biotin-Konjugat Conjugué Biotine Coniugato biotinilato Conjugado de Biotina
EL	Enzyme Label Enzym-Marker Marqueur enzymatique Marcato enzimatico Marcador enzimático
SUBS TMB	TMB Substrate TMB-Substrat Substrat TMB Substrato di TMB Substrato de TMB
SOLN STOP	Stop Solution Stopp-Lösung Solution stop Soluzione stoppante Solución de interrupción

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