6-Sulfatoxymelatonin

ELISA

For research use only.
Not for use in diagnostic procedures.

EK-M6S-U 96 tests

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INTENDED USE

The 6-Sulfatoxymelatonin ELISA Kit provides materials for the direct and quantitative determination of 6-sulfatoxymelatonin (6-SMT) in human and animal urine (1-7).
For research use only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The 6-SMT ELISA is a competitive immunoassay using a capture antibody technique (8). A polyclonal antibody specific for rabbit immunoglobulin has been coated onto the microtiter plate provided in the kit. During the first 3-hours incubation, 6-SMT present in the pre-diluted urine samples, Controls and ready to use Calibrators, respectively, compete with biotinylated 6-SMT for the binding sites of a highly specific rabbit anti-6-SMT antibody, while the formed (biotinylated) 6-SMT-antibody complexes are captured by the second antibody coated on the wells. After washing, the Enzyme Label (streptavidin conjugated to horseradish peroxidase, HRP) is added which binds during a second 30-minutes incubation step to the 6-SMT-biotin-antibody complexes captured on the coated wells. Unbound Enzyme Label is then removed by a second washing step and TMB (Tetramethylbenzidine) substrate is added to the wells. In a third 15-minutes incubation step, a colored product is formed in inverse proportion to the amount of 6-SMT originally 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	Quantities	Code	Reconstitution
Microtiter Plate precoated with goat anti-rabbit Ig	8x12 wells	B-M6S-MP	Wash 2x before use
Plate Sealer	3 pcs.		
Wash Buffer Concentrate (10x) with preservatives	1 bottle 100 ml	B-WB	Dilute with 900 ml of deionized water
Incubation Buffer with preservatives	1 bottle 100 ml	B-M6S-IB	Ready to use
Calibrators A to F[1)] 6-SMT in a buffer matrix with preservatives	1 vial 2 ml 5 vials 0.5 ml	B-M6S-CASET	Ready to use
Control Low / High[2)] Diluted human urine with preservatives	2 vials 0.5 ml	B-M6S-CONSET	Ready to use
Antiserum Rabbit anti-6-SMT in a buffer matrix with preservatives	1 vial 5.5 ml	B-M6S-AS	Ready to use (yellow solution)
Biotin Conjugate 6-SMT conjugated to biotin in a buffer matrix with preservatives	1 vial 5.5 ml	B-M6S-BC	Ready to use (blue solution)
Enzyme Label Streptavidin-HRP in a protein-based buffer with preservatives	1 vial 11 ml	B-M6S-EL	Ready to use (yellow solution)
TMB Substrate Citrate buffered with hydrogen peroxide	1 vial 11 ml	B-TMB	Ready to use (colourless)
Stop Solution 0.25 M sulfuric acid	1 vial 11 ml	B-STS	Ready to use **Irritant**

Table 1

[1)] The Calibrator A is the Zero Calibrator and does not contain 6-SMT (2 ml/vial). Calibrators B, C, D, E and F effectively contain 4, 10, 25, 62.5 and 200 pg/ml of 6-SMT, respectively (0.5 ml/vial). As the recommended dilution for urine samples is 1 in 200, the Calibrators B, C, D, E and F are labeled as follows: 0.8, 2, 5, 12.5 and 40 ng/ml, respectively. In this way, the sample dilution is already taken into account for the final result calculations.

[2)] The Controls contain lot-specific amounts of 6-SMT. Refer to the QC Data Sheet for the exact concentrations.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
All unopened kit components are stable at 2-8°C until the expiration date printed on the labels.	
Opened / Reconstituted Reagents	
Microtiter Plate	Return unused strips to the aluminum pouch and reseal along the entire edge of zip-seal. Store for up at 2-8°C until expiration date printed on the labels.
Wash Buffer	Store at 2-8°C until expiration date printed on the labels.
Incubation Buffer	
Calibrators	
Controls	
Antiserum	
Biotin Conjugate	
Enzyme Label	
Substrate Solution	
Stop Solution	Store at 18-28°C until expiration date printed on the label.

Table 2

PRECAUTIONS

SAFETY PRECAUTIONS

- This test is for in vitro research use only, and must be handled by qualified personnel, in accordance with good laboratory practices (GLP).
- The Calibrators (B-M6S-CASET) and the Controls (B-M6S-CONSET) of this kit contain components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with good laboratory practices using appropriate precautions.
- The Stop Solution (B-STS) contains sulfuric acid. It is irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. After contact with eyes or skin, wash immediately with plenty of water.
- Unused solution should be disposed of according to local, state and federal regulations.

TECHNICAL PRECAUTIONS

Kit components

- Read carefully the instructions prior to 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.
- Concentrated wash buffer may contain salt crystals. Make sure these crystals have completely dissolved after dilution of the concentrate by stirring the diluted buffer at ambient temperature and then refrigerate before usage in the assay.
- If an automated plate washer is used**,** “plate mode” should be chosen so that dispensing is performed sequentially on all strips before aspirating.
- Components must not be used after 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.
- The enzyme used as the label is inactivated by oxygen and is highly sensitive to sodium azide, thimerosal, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Therefore, use only deionized high quality water.

Assay Procedure

- 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.
- If the initial concentration of a sample exceeds the concentration of the highest calibrator, the urine sample should be further diluted with Incubation Buffer and assayed again according to the assay procedure. The additional dilution must be considered when calculating the actual concentration of 6-SMT present in the sample.
- If the initial concentration of a sample is lower than the lowest concentration of the calibrator, the urine sample should be less diluted with Incubation Buffer (e.g. by a factor of 20 instead of 200) and assayed again according to the assay procedure. The lower dilution factor must be considered when calculating the actual concentration of 6-SMT present in the sample.

EQUIPMENT REQUIRED

- Precision pipettes with disposable tips: 5 µl, 50 µl, 100 µl and 1 ml pipettes.
- Microtiter plate washer or squeeze bottle for Wash Buffer.
- Refrigerator.
- Microtiter plate rotator.
- Microtiter plate reader for measurement of absorbance at 450 nm.

MATERIALS RECOMMENDED BUT NOT PROVIDED

- Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions.
- 1000 ml cylinder for the dilution of the Wash Buffer Concentrate.
- Blotting paper.

SPECIMEN COLLECTION AND STORAGE

The procedure calls for <10 µl of urine. Collect urine, centrifuge for 1 minute at 10,000 x g or 5 minutes at 2000 x g and transfer aliquots into fresh microtubes. Urinary 6-SMT is stable for several weeks even at ambient temperatures. However, due to potential growth of microorganisms it is recommended to store the urine samples at ≤-20°C. Samples are stable for at least 18 months if stored at ≤-20°C. Avoid repeated freeze-thaw cycles. Frozen samples should be thawed and mixed thoroughly by vortexing prior to use.

ASSAY PROCEDURE

1. Dilute all patient urinary samples 1:200 with Incubation Buffer (e.g. 5 µl of urine + 1 ml of Incubation Buffer).
2. Prepare a plate with sufficient strips to test the desired number of Calibrators, Controls and samples. Remove excess strips from the holder and reseal them in the foil pouch. Store refrigerated.
3. Empty the wells and wash the coated wells twice using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

4a. Pipet 100 µl of Calibrator A in duplicate into wells A1+A2 (Blank wells).

4b. Pipet 50 µl of Calibrator A (Zero Standard) in duplicate into wells B1+B2.
Pipet 50 µl of Calibrator B in duplicate into wells C1+C2.
Pipet 50 µl of Calibrator C in duplicate into wells D1+D2.
Pipet 50 µl of Calibrator D in duplicate into wells E1+E2.
Pipet 50 µl of Calibrator E in duplicate into wells F1+F2.
Pipet 50 µl of Calibrator F in duplicate into wells G1+G2.
Pipet 50 µl of Low Control in duplicate into wells H1+H2.
Pipet 50 µl of High Control in duplicate into wells A3+A4.

4c. Pipet 50 µl of each diluted sample in duplicate into the subsequent wells.

5. Add 50 µl of Biotin Conjugate (blue solution) to all wells.
6. Add 50 µl of Antiserum (yellow solution) to all wells, **except Blank wells** (wells A1+A2). Cover the plate with a plate sealer and place it for 60 seconds on a plate rotator set at 600 rpm.
7. Incubate for 3 hours (± 5 min) at 2-8°C.
8. Remove and discard the Plate Sealer. Empty the wells and wash four times using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
9. Add 100 µl of Enzyme Label (yellow solution) to all wells.
10. Cover the plate with a Plate Sealer and incubate for 30 minutes (± 5 min) at 2-8°C.
    Important: Allow the TMB substrate solution to reach 18-28°C.
11. Remove and discard the Plate Sealer. Empty the wells and wash four times using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
12. Add 100 µl of the TMB Substrate Solution to each well.
13. Cover the plate with a Plate Sealer, place the plate on a plate rotator set at 600 rpm, protect the plate from direct light and incubate for 30 minutes (± 2 min) at 18-28°C.
14. Add 100 µl of Stop Solution to all wells. Remove air bubbles with a pipette tip. Proceed to step 15. within 30 minutes.
15. Read the absorbance at 450 nm in a microtiter plate reader.

RESULTS & STANDARDIZATION

Standard Curve: Record the absorbance at 450 nm for each calibrator and blank (NSB) well. Average the duplicate values, subtract the average of the blank wells (NSB) 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 NSB-corrected absorbance of the Zero Calibrator taken as 100 %.

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

Plot the percent bound (vertical axis) versus the concentration of 6-SMT in ng/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 similar algorithm.

Samples and Controls: Record the absorbance at 450 nm for each sample well. Average the duplicate values, subtract the average of the blank wells and record the averages (=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 NSB-corrected absorbance of the 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 6-SMT concentration (ng/ml) from the horizontal axis.
See Table 3 and Figure 1 for examples of results and standard curve.

Standardization: The NovoLytiX 6-Sulfatoxymelatonin ELISA is calibrated against a certified Reference Standard (Toronto Research Chemicals # S689050; CAS 2208-40-

4), and its correct concentration used to generate the kit Calibrators was confirmed by UV/VIS:

ε_{222} = 39'727 $M^{-1}cm^{-1}$ in H_2O.

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 using precise laboratory techniques (current GLP guidelines) and accurately following this instruction for use.

Since there are no controls for urinary 6-SMT commercially available, we recommend to use urine pools containing different levels of 6-SMT for internal quality controls. 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 QC data sheet.

The accuracy of each actual calibrator lot is assured by comparison against a certified Reference Standard (Toronto Research Chemicals # S689050; CAS 2208-40-4).

If the precision of the assay does not correlate with the established limits and repetition excludes 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; vi) purity of water.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (Limit of Blank, LoB): 0.14 ng/ml. 24 duplicates of Zero Calibrator (Calibrator A) were assayed in a single run. Mean and standard deviation were calculated for the absorbance values. The minimum detectable dose of 6-SMT was calculated to be 0.14 ng/ml by substracting two standard deviations from the mean absorbance of the Zero Calibrator (Calibrator A) and intersecting this value with the standard curve obtained in the same run.

Functional Sensitivity (Limit of Quantitation, LoQ): 1.5 ng/ml. The Limit of Quantification (LoQ) is defined as the minimum 6-SMT concentration in urine that can be measured with an inter-assay coefficient of variation (CV) of less than 15%. The LoQ was determined from 7 different urinary samples each measured in one duplicate pair of tubes over 10 assays. The LoQ was calculated to be 1.5 ng/ml (at a sample dilution of 1:200).

Intra-Assay Precision (Within-Run): 7.1%. The intra-assay precision was calculated from the results of 24 pairs of values obtained in a single run from three urine samples containing different concentrations of 6-SMT. The results are presented in Table 4.

Inter-Assay Precision (Run-to-Run): 11.9%. From 6 urine samples containing different concentrations of 6-SMT the inter-assay precision was calculated from the results of 10 pairs of values obtained in 10 different runs. The results are presented in Table 5.

Dilution Linearity/Parallelism: 97.8%. Three urine samples containing high concentrations of 6-SMT were sequentially diluted with Incubation Buffer and assayed according to the assay procedure. The results are presented in Table 6.

Spiking Recovery: 119%. Three human urine samples were spiked with increasing amounts of 6-SMT and assayed according to the assay procedure. The results are presented in Table 7.

Specificity: The cross-reactions of the rabbit anti-6-SMT antibody have been determined at 50% binding. The results are presented in Table 8.

Method Comparison: 42 urine samples were analyzed using the 6-Sulfatoxymelatonin ELISA versus a commercially available reagent set for measuring 6-SMT by means of an ^{125}I-radioimmunoassay which is often used and cited in the scientific literature (*e.g.* 9-11). The linear regression analysis of the data yielded the following statistics (see Figure 2):

6-SMT ELISA = 0.75 x RIA +1.70 ng/ml; R^2 = 0.984.

Table 3: **Example of Results**

	Conc. (ng/ml)	Absorbance (OD)	B/B_0 (%)	Calc. Conc. (ng/ml)	CV Conc. (%)
Blank		0.127			
Blank		0.119			
Blank Avg.		**0.123**			**4.6**
Cal. A	0.0	2.213	100.0		
Cal. A	0.0	2.176	100.0		
Cal. A Avg.	**0.0**	**2.194**	**100.0**		**1.2**
Cal. B	0.8	1.960	88.7	0.8	
Cal. B	0.8	1.966	89.0	0.8	
Cal. B Avg.	**0.8**	**1.963**	**88.8**	**0.8**	**2.1**
Cal. C	2.0	1.699	76.1	2.0	
Cal. C	2.0	1.705	76.4	2.0	
Cal. C Avg.	**2.0**	**1.702**	**76.2**	**2.0**	**1.0**
Cal. D	5.0	1.205	52.2	4.9	
Cal. D	5.0	1.192	51.6	5.1	
Cal. D Avg.	**5.0**	**1.199**	**51.9**	**5.0**	**1.5**
Cal. E	12.5	0.719	28.8	12.2	
Cal. E	12.5	0.697	27.7	12.8	
Cal. E Avg.	**12.5**	**0.708**	**28.2**	**12.5**	**3.7**
Cal. F	40.0	0.365	11.7	40.0	
Cal. F	40.0	0.365	11.7	40.0	
Cal. F Avg.	**40.0**	**0.365**	**11.7**	**40.0**	**0.0**
Ctrl. LOW		1.485		3.1	
Ctrl. LOW		1.444		3.3	
Ctrl. L. Avg.		**1.464**		**3.2**	**5.1**
Ctrl. HIGH		0.593		17.0	
Ctrl. HIGH		0.579		17.7	
Ctrl. H. Avg.		**0.586**		**17.3**	**3.0**
Sample 1		0.808		10.1	
Sample 1		0.794		10.3	
Sam. 1 Avg.		**0.801**		**10.2**	**2.0**
Sample 2		0.392		35.5	
Sample 2		0.411		32.8	
Sam. 2 Avg.		**0.401**		**34.2**	**5.6**

ED_{20} = 20.2 ng/ml ED_{50} = 5.3 ng/ml ED_{80} = 1.6 ng/ml

These results and standard curve are for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

Figure 1: **Example of a Standard Curve**

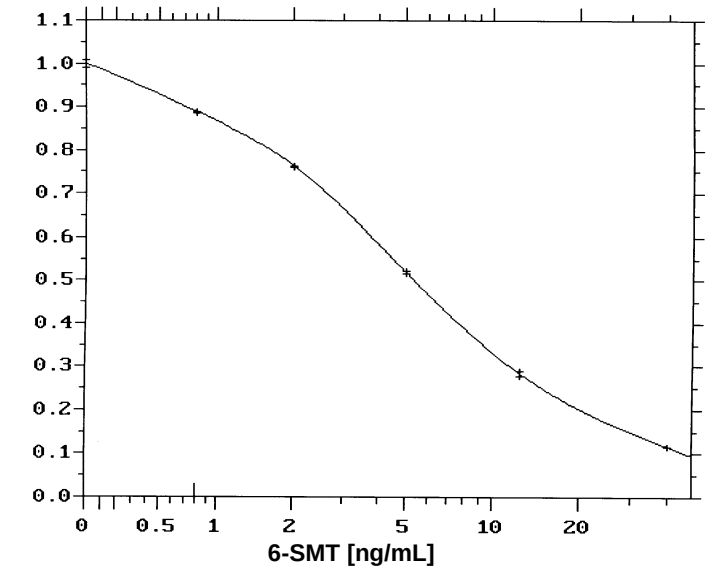


Table 4: **Intra-Assay Precision**

Urine Sample, diluted 1:200	Mean (ng/ml)	S.D. (ng/ml)	C.V. (%)
1	3.09	0.30	9.7
2	11.29	0.70	6.2
3	34.65	1.82	5.3
Mean			**7.1%**

Table 5: **Inter-Assay Precision**

Urine Sample diluted 1:200	Mean (ng/ml)	S.D. (ng/ml)	C.V. (%)
5	1.6	0.28	17.4
6	1.5	0.24	15.3
7	2.0	0.27	13.2
8	3.2	0.30	9.6
9	10.6	0.79	7.5
10	32.1	2.71	8.4
Mean			**11.9%**

Table 6: **Dilution Linearity/Parallelism**

Sample	Basic Value (ng/ml)	Dilution Factor	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
11	29.7	1:200	29.7	---	----
		1:400	15.2	14.8	102
		1:800	7.3	7.4	98
		1:1600	3.7	3.7	100
		1:3200	2.0	1.9	110
12	26.7	1:50	26.7	--	--
		1:100	13.3	13.4	100
		1:200	6.4	6.7	96
		1:400	3.2	3.3	96
		1:800	1.5	1.7	90
		1:1600	0.8	0.8	92
13	16.9	1:12.5	16.9	--	--
		1:25	7.5	8.5	89
		1:50	3.8	4.2	89
		1:100	2.1	2.1	97
		1:200	1.2	1.1	112
Mean					**97.8%**

Table 7: **Spiking Recovery**

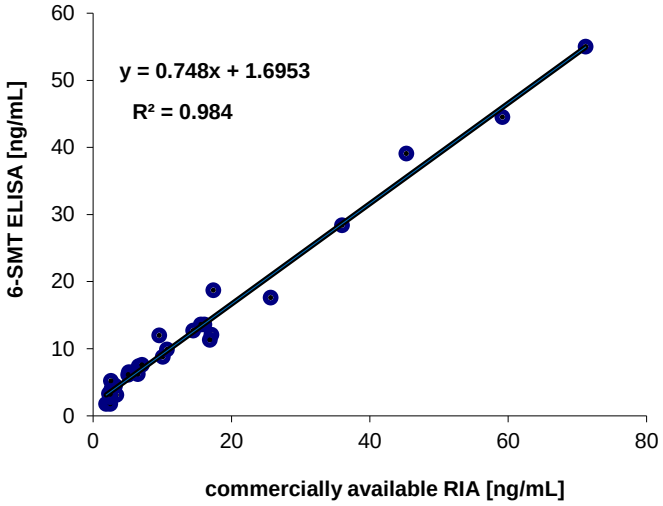
Sample	Basic Value (ng/ml)	Spiked with (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
14	0.9	0.5	1.4	1.4	104
		1.0	1.9	1.9	100
		2.0	2.9	3.5	123
		4.0	4.9	3.8	77
		8.0	8.9	8.6	97
		16.0	16.9	17.3	103
		32.0	32.9	28.1	85
15	6.3	0.5	6.8	6.2	91
		1.0	7.3	6.9	94
		2.0	8.3	7.9	95
		4.0	10.3	10.3	100
		8.0	14.3	17.8	124
		16.0	22.3	28.2	127
		32.0	38.3	38.2	100
16	4.6	0.5	5.1	4.0	79
		1.0	5.6	5.2	92
		2.0	6.6	7.6	115
		4.0	6.6	10.4	122
		8.0	12.6	14.8	118
		16.0	20.6	25.8	125
		32.0	36.6	35.8	98
Mean					**119%**

Table 8: **Specificity**

6-Sulfatoxymelatonin	100 %
N-Acetyl-Serotonin Sulfate	0.01 %
Melatonin	0.007 %
6-Hydroxymelatonin	0.001 %
Related compounds as follows:	< 0.001 %

5-Sulfatoxy-N-Acetylserotonin, 5-Glucuronide-N-Acetylserotonin, N-Acetylserotonin, 6-Glucuronidemelatonin, 5-Methoxyindole Acetic Acid, Tryptophan, N-Acetyltryptophan, 5-Methoxytryptophan, 5-Hydroxytryptophol, N-Acetyltryptamine, N-Methyltryptamine, 5-Hydroxytryptamine, 5-Methoxytryptamine.

Figure 2: **Method Comparison**



APPENDIX II

REFERENCES

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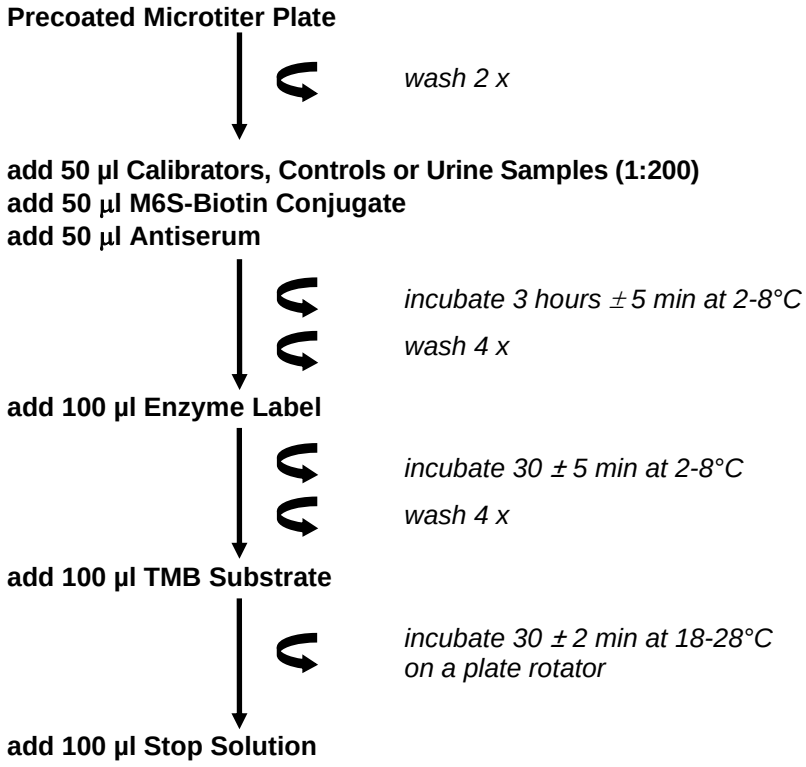
6-SULFATOXYMELATONIN ELISA



TIME TO RESULT: 4.5 HOURS

Date	Version	Reason for change
2021-12-08	01	1st tracked NovoLytiX version.
2022-03-26	02	Microtiter Plates are delivered with wells containing 250µL of stabilization buffer instead in a lyophilized form, this IFU is accordingly adapted; the former section *MATERIALS REQUIRED BUT NOT PROVIDED* is divided into sections *EQUIPMENT REQUIRED* and *MATERIALS RECOMMENDED BUT NOT PROVIDED*, starting with lot 3738; a procedural note is added to step 13 of the *ASSAY PROCEDURE* (incubation with TMB): If the color reaction is slow or not as high as expected the incubation with TMB may be prolonged for another 15 minutes; the accuracy of each actual calibrator lot is assured by comparison against a certified Reference Standard (Toronto Research Chemicals # S689050; CAS 2208-40-4) (see section *QUALITY CONTROL*).
2023-07-06	03	Change of Wash Buffer 10x code from B-M6S-WB to B-WB; down rating of B-STS from corrosive agent to irritant according to the latest REACH guidelines; the formulation of TMB solution has been slightly changed (without affecting its performance) and is therefore not anymore regarded irritant according to the latest REACH guidelines.
2024-03-19	04	The use of refrigerated reagents and refrigerated Wash Buffer is not anymore required (see *PRECAUTIONS* and *ASSAY PROCEDURE, steps 3 to 9*) without affecting the assay performance or the final results; the standardization of the 6-Sulfatoxymelatonin ELISA and some other statements are clearer formulated; several typing errors are erased.
2024-04-13	04.1	Intended use extended to animal urine; *ASSAY PROCEDURE, step. 13 (incubation with TMB Solution)* new for 30±2 min to reach higher OD (optical density) values in general.

APPENDIX V
SYMBOLS

Symbol	Explanation
	Use by Verwendbar bis Utiliser jusqu'au Utilizzare entro Fecha de caducidad
REF	Catalogue number Bestellnummer Référence du catalogue Numero di catalogo Número de catálogo
LOT	Batch code Chargenbezeichnung 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*
	Content sufficient for <n> tests Ausreichend für "n" Ansätze Contenu suffisant pour „n" tests Contenuto sufficiente per „n" saggi Contenudo sufficiente 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
MP	Microtiterplate Mikrotiter-Platte Microplaque Micropiastra Microplaca
BUF WASH 10X	Wash Bufer Concentrate (10x) Wasch-Puffer Konzentrat (10x) Concentré de tampon de lavage (10x) Tampone di lavaggio concentrato (10x) Tampón de lavado concentrado (x10)

Symbol	Explanation
BUF INC	Incubation Buffer Inkubations-Puffer Tampon d'incubation Tampone d'incubazione Tampón de incubación
CAL A - CAL F	Calibrator A -F Kalibrator A -F Calibrateur A -F Calibratore A - F Calibrador A - F
CONTROL L	Control Low Kontrolle tief Contrôle bas Controllo basso Control bajo
CONTROL H	Control High Kontrolle hoch Contrôle élevé Controllo alto Control alto
Ab	Antiserum Antiserum Antisérum Antisiero Antisuero
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 parada