MELATONIN

EXTRACTION SET

MLTN-EXSET 80 extractions

Revision date: 2022-02-14

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INTENDED USE

The Melatonin Extraction Set is intended for the quantitative extraction of melatonin from serum, plasma, urine and other biological specimens upon C18 solid phase extraction.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Extraction Columns[1)] C18 reversed phase extracti columns	20 pcs.	B-MEC	
Incubation Buffer with preservatives	1 vial 25 ml	MLTN-IB	Ready to use
Control Low / High[2)] melatonin in human base matrix; with preservatives	1 set of 2 vials 1.5 ml	B-MEL-CONSET15	Ready for extraction

Table 1

1) Each extraction column provided with this kit can be utilized up to five times if used according to the extraction procedures described in this instruction for use.

2) Lot specific amount of melatonin: see QC Data Sheet added to the kit. Extract the Controls according to the protocol as described on below.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened reagents	
Store the reagents as indicated on the respective labels. Do not use past kit expiration date.	
Opened / reconstituted reagents	
Extraction Columns	Used columns should be stored at 18-28°C and protected from dust and light.
Incubation Buffer	Store at 2-8°C until expiration date printed on the label.
Controls	Store at -20°C until expiration date printed on the labels.

Table 2

WARNINGS AND PRECAUTIONS

SAFETY PRECAUTIONS

- The Controls (B-MEL-CONSET15) 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.

TECHNICAL PRECAUTIONS

- 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.
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.

MATERIALS REQUIRED BUT NOT PROVIDED

- 100–1000 µl precision pipette (or preferably an adjustable multipipette) with disposable tips
- Disposable borosilicate glass tubes for the preparation of extracts (*e.g.* disposable CW glass test tubes from Baxter; no. 451296)
- Extraction vacuum manifold for applying the extraction columns; alternatively, the extraction procedure can also be performed using a centrifuge (see below)
- Methanol *(HPLC grade)*
- Hexane *(p.a.)*
- Deionized double distilled water (ultrapure; not containing any organic residues)
- Vacuum concentrator or supply for particle free nitrogen
- Vortex mixer

SPECIMEN COLLECTION AND STORAGE

When drawing blood at night, a dim flash light or a yellow light (≤ 100 lux) should be used in order to avoid a possible light influence on the melatonin profile.

Serum: The procedure calls for about 0.5 ml of blood or for 0.25 ml of serum per extraction (if the sample is not diluted after extraction). Collect blood into plain tubes, avoid hemolysis, leave to clot for 45 min at room temperature (18-28°C) protected from light. Centrifuge at 3000 x g for 10 min at room temperature and collect the serum. Lipemic, hemolytic and icteric samples should not be used in this assay. Lipemic samples can be avoided by asking patients to fast for at least 12 hours prior to blood drawing.

Plasma: The procedure calls for about 0.5 ml of blood or for 0.25 ml of plasma per extraction (if the sample is not diluted after extraction). Collect blood into EDTA or Heparin tubes, centrifuge for 10 min at 3000 x g for 10 min at room temperature and collect the plasma. Do not use grossly hemolysed samples.

Specimen Storage: If not extracted immediately, serum or plasma samples should be frozen and can be stored for at least 12 months at -20°C. Repeated freeze-thaw cycles should be avoided.

EXTRACTION OF SAMPLES AND CONTROLS

- Each extraction column provided with this kit can be used up to five times according to the extraction procedures described below. They should be stored at 18-28°C and protected from light and dust.
- Always use HPLC GRADE METHANOL AND HEXANE as well as DEIONIZED WATER OF ULTRAPURE QUALITY (no organic residues such as oils or detergents) for the extraction procedure.
- In order to avoid clogging of the columns, FILTER OR CENTRIFUGE SAMPLES CONTAINING PARTICLES such as fibrin clots prior to the extraction (*e.g.* heparin plasma that was frozen).
- If samples have to be measured containing >50 pg/ml of melatonin, the sample volume may be reduced down to 0.125 ml without a notable change in extraction recovery. Alternatively, the dried extract may be reconstituted with higher volumes than the recommended volume of 0.25 ml.
- The extraction PROCEDURE was TESTED AND VALIDATED FOR HUMAN SERUM, PLASMA, SALIVA AND URINE samples. If it is intended to measure another specimen, we recommended to validate the extraction recovery using melatonin-spiked specimens.

Extraction Procedure using Centrifugation

COLUMN PREPARATION & CONDITIONING

- Mark 1 extraction column for each sample to be extracted and place them into polypropylene or glass tubes.
- Add 1 ml of methanol to columns, centrifuge for 1 min at 200 x g. Repeat this step once.
- Add 1 ml of H_2O to columns, centrifuge for 1 min at 200 x g. Repeat this step once.
- Proceed with sample application without delay.

SAMPLE APPLICATION

- Add 0.2 ml of sample to the correspondingly marked column, centrifuge for 1 min at 200 x g.

WASHING

- Add 1 ml 10% methanol in H_2O (v/v) to the columns, centrifuge for 1 min at 500 x g. Repeat this step once.
- Add 1 ml of hexane to columns, centrifuge for 1 min at 500 x g.

ELUTION OF EXTRACT

- Place the columns into clean correspondingly marked borosilicate tubes.
- Add 1 ml of methanol to columns, centrifuge for 1 min at 200 x g.
- Use column for extracting the next sample (up to 5 times) or store column at 18-28°C and protected from light and dust.

EVAPORATION & RECONSTITUTION OF EXTRACT

- Evaporate the methanol to dryness using a vacuum concentrator with a cold trap. Alternatively, use a 37°C heating block or water bath and evaporate the methanol to dryness with a stream of particle free nitrogen.
- Reconstitute the samples with 0.25 ml of Incubation Buffer, vortex.
- Equilibrate the extracts for 30 min at 18-28°C, vortex. Store reconstituted extracts capped and frozen if not assayed immediately.

Extraction Procedure using Vacuum Manifold

Note: If not indicated otherwise, always pass the solvent through the column using vacuum and a flow rate of ≤ 5 ml/min.

COLUMN PREPARATION & CONDITIONING

- Mark 1 extraction column for each sample to be extracted and place them into polypropylene or glass tubes.
- Add 2 x 1 ml of methanol to columns, let the solvent pass through using vacuum.
- Add 2 x 1 ml of H_2O to columns, let the solvent pass through using vacuum.
- Proceed with sample application before the column gets dry.

SAMPLE APPLICATION

- Add 0.2 ml of sample to the correspondingly marked column, let the solvent pass through slowly (≤ 2 ml/min).
- Proceed with washing before the column gets dry.

WASHING

- Add 2 x 1 ml of 10% methanol in H_2O (v/v) to columns, let the solvent pass through using vacuum.
- Add 1 ml of hexane to the columns, let the solvent pass through using vacuum.
- Apply vacuum for 1 more min in order to evaporate remaining hexane in the column.

ELUTION OF EXTRACT

- Place the columns into clean correspondingly marked borosilicate tubes.
- Add 1 ml of methanol to columns, let the solvent pass through slowly using vacuum and a flow rate of ≤ 2 ml/min.
- Use column for extracting the next sample (columns can be used up to 5 times) or store column at 18-28°C and protected from light and dust.

EVAPORATION & RECONSTITUTION OF THE EXTRACT

- Evaporate methanol to dryness using a vacuum concentrator with a cold trap. Alternatively, use a 37°C heating block or water bath and evaporate the methanol to dryness with a stream of particle free nitrogen.
- Reconstitute the samples with 0.25 ml of Incubation Buffer, vortex.
- Equilibrate the extracts for 30 min at 18-28°C, vortex. Store reconstituted extracts capped and frozen if not assayed immediately.

MELATONIN DETERMINATION IN/OF THE EXTRACTS

- The reconstituted extracts can be assayed in the NovoLytiX Melatonin ELISA (order code: **MLTN-96**). Please read the respective **Instructions for Use** carefully.
- The NovoLytiX Melatonin ELISA calls for 100 µl of reconstituted extract per microtiter well.
- The **dilution factor of 1.25** (200 µl sample volume applied to extraction columns; extracts reconstituted in 250 µl of Incubation Buffer) is considered in the concentration specifications of Calibrators A, B, C, D and E of the MLTN-96 ELISA which contain the following melatonin concentration: 0.4, 1.2, 4, 12 and 40 pg/mL, but are labeled with 0.5, 1.5, 5, 15, and 50 pg/mL of melatonin, respectively.

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.

The reproducibility of 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 added to this kit.

If the precision of the exraction 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) expiration dates of reagents iii) storage and incubation conditions iv) purity of water.

LIMTATIONS

Melatonin results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Precision of Column Extraction: 8.2%. A daytime human serum was extracted 12 times in parallel using 12 separate extraction columns. Subsequently, all extracts were analyzed in a single assay run according to the assay procedure of the NovoLytiX Melatonin RIA (order code: RK-MEL2).

Sample	Mean [pg/ml]	SD [pg/ml]	CV [%]
Low Serum1	1.6	0.22	14.0
Low Serum2	2.8	0.17	6.1
High Serum	19.5	0.88	4.5
Mean			**8.2**

Table 3

Extractive Dilution Linearity: 108.8 %. Varying amounts of a human serum sample containing an elevated concentration of melatonin were applied onto extraction columns, extracted according to the protocol and subsequently assayed according to the assay procedure of the NovoLytiX Melatonin RIA (order code: RK-MEL2).

Sample	Volume applied [ml]	Obs. (O) [pg/ml]	Exp. (E) [pg/ml]	% O/E
High Serum	1	29.1	-	-
	0.5	15.1	14.6	103.8
	0.25	7.8	7.3	107.2
	0.125	4.2	3.6	115.4
Mean				**108.8**

Table 4

Extraction Recovery: 99.9 %.

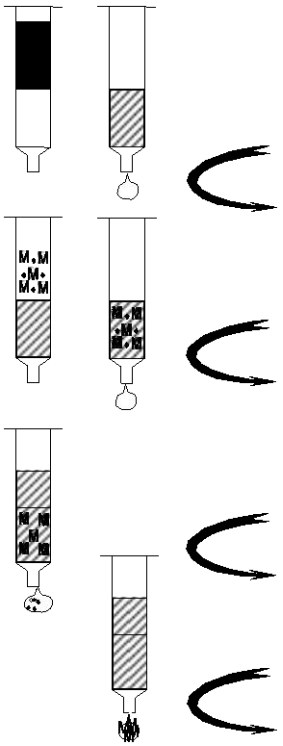
Two serum samples were spiked with increasing amounts of melatonin, extracted and analyzed according to the assay procedure of the NovoLytiX Melatonin RIA (order code: RK-MEL2).

Sample	Basic Value	Added [pg/ml]	Observed [pg/ml]	Expected [pg/ml]	Recovery [%]
A	0.46	1	1.2	1.5	83
		2	2.2	2.5	88
		5	5.2	5.5	95
		10	10.5	10.5	100
		20	21.7	20.5	106
		40	41.0	40.5	101
B	0.61	1	1.9	1.6	120
		2	2.3	2.6	87
		5	5.5	5.6	98
		10	11.8	10.6	111
		20	22.1	20.6	107
		40	41.3	40.6	102
Mean					**99.9**

Table 5

EXTRACTION PROCEDURE

C18 column



Conditioning

2 x 1 ml methanol
2 x 1 ml water

aspirate or centrifuge

Load column

0.2 ml of sample

aspirate or centrifuge

Wash column

2 x 1 ml 10% (v/v) methanol
1 ml hexane

aspirate or centrifuge

Elute Melatonin

1 ml methanol

aspirate or centrifuge

Evaporate to dryness
and reconstitue in 0.25 ml
of Incubation Buffer

Symbol	Explanation
	Use by
REF	Catalogue number
LOT	Batch code
	Temperature limitation
	Consult Instructions for Use
Σ	Contains sufficient for <n> extractions

Symbol	Explanation
MEC	Extraction Column
BUF INC	Incubation Buffer
CONTROL L	Control Low
CONTROL H	Control High

CE

IVD

Printing date:
2022-02-16