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Instructions for use

Serotonin ELISA Fast Track

REF BA E-8900

2°C 8°C Σ 96 IVD CE

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1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of serotonin in urine and serum, to evaluate serotonin homeostasis. The determination of serotonin in urine is helpful for the assessment of neurostress.
The quantitative determination of serotonin follows the basic principles of the enzyme immunoassay.
In the first step, serotonin is quantitatively acylated. The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples compete with the solid phase bound analytes for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user. This in-vitro diagnostic is for professional use only.

1.2 Clinical application

Serotonin (5-hydroxytryptamine) is an intermediate product of tryptophan metabolism [1], a well-studied neurotransmitter, and may also act as a peripheral hormone [2]. Synthesis occurs mainly in enterochromaffin cells (ec-cells) of the gastrointestinal tract and in neurons [1, 3]. It is present in high concentrations in ec-cells of the intestine, serotonergic neurons of the brain, and platelets [1, 3-6]. Serotonin is mainly degraded to 5-hydroxyindole acetic acid (5-HIAA) or melatonin [1, 7] and can be excreted in the urine [8]. In the bloodstream, the vast majority of serotonin is found in platelets [9] and can be readily detected in serum [1, 10, 11]. Altered serotonin levels in serum and/or urine can indicate both physical and psychological dysfunction.
Serotonin balance may be impaired in serum and/or urine in a variety of conditions. For example, decreased serotonin levels have been demonstrated in depression, anxiety, and even pain sensitivity compared to unaffected subjects [6, 8, 10]. Increased serotonin levels, on the other hand, have been reported in patients with serotonin-secreting neuroendocrine tumors, also called carcinoid tumors [2, 12, 13], or hepatocellular carcinomas [14].
Therapeutic consequences should never be based on laboratory results alone, even if these results are assessed in accordance with the quality criteria of the method. Any laboratory result is only a part of the total clinical picture of the patient.
Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient, it can be used for therapeutic consequences.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for a certain type of sample as indicated in Intended Use (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) must be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) If serious incidents should occur in connection with this product, they should be reported to the manufacturer and the competent national authorities.
(6) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
(8) Standards, Controls and specimen samples should be assayed in duplicate.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
(10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(15) For information about hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.

(16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(17) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(18) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.
(19) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but must be correlated to other diagnostic tests and clinical observations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.
If you have any further questions, please contact the manufacturer.

2.2.1 Interfering substances and proper handling of specimens

Urine
Please note the sample collection! It cannot be excluded that high acid concentrations lead to incorrect results. Up to 30 µl 100% acetic acid per 1 ml urine no influence on the results was observed.

Serum
Samples containing precipitates or fibrin strands might cause inaccurate results.
Hemolytic samples (up to 2 mg/ml hemoglobin), icteric samples (up to 50 mg/dl bilirubin) and lipemic samples (up to 834 mg/dl triglycerides) have no influence on the assay results.
If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

2.2.2 Drug and food interferences

The following foods and stimulants can affect the serotonin content in the sample. Alcohol, pineapple, eggplant, avocados, bananas, grapefruit, currants, cocoa, kiwis, caffeine, melons, mirabelles, nicotine, pecans, peaches, plums, chocolate, gooseberries, tomatoes, walnuts.
Some drugs can also affect serotonin levels in the sample. For example, taking amphetamines, acetanilide, coumarins, ephidrine, guaifenesin, mephenesin (carbamate), methocarbamol, monoamine oxidase inhibitors (MAO inhibitors), acetaminophen, phenacetin, phenobarbital, phentolamine, or reserpine can lead to increased serotonin levels. In contrast, acetylsalicylic acid, chlorpromazine, isoniazid, levodopa, methenamine, methyldopa, promethazine, selective serotonin reuptake inhibitors (SSRIs), or streptozocin may result in decreased serotonin levels.
Therefore, 2 – 4 days prior to specimen collection, these foods should be avoided and the medications discontinued if medically justifiable.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazardous ingredients:	2-methyl-2H-isothiazol-3-one	

Hazard statements:	H317 May cause an allergic skin reaction.
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.

BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	
BA E-0931	**IU SER 5-HIAA**	**Serotonin Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable white pouch with desiccant	
BA E-6612	**ACYL-REAG**	**Acylation Reagent** – ready to use
Content:	Acylation reagent in DMSO	
Volume:	2 x 3 ml/vial, white cap	
BA E-8910	**SER-AS**	**Serotonin Antiserum** – ready to use
Content:	Rabbit anti-Serotonin antibody, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species is rabbit	
BA E-8911	**ACYL-BUFF**	**Acylation Buffer** – ready to use
Content:	TRIS buffer with non-mercury preservative	
Volume:	1 x 55 ml/vial, grey cap	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/Cap	Concentration [ng/ml] (= µg/l)	Concentration [nmol/l]	Volume/ Vial
BA R-8901	**STANDARD A**	white	0	0	4 ml
BA R-8902	**STANDARD B**	yellow	15	85	4 ml
BA R-8903	**STANDARD C**	orange	50	284	4 ml
BA R-8904	**STANDARD D**	blue	150	851	4 ml
BA R-8905	**STANDARD E**	grey	500	2,840	4 ml
BA R-8906	**STANDARD F**	black	2,500	14,175	4 ml
BA R-8951	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA R-8952	**CONTROL 2**	red			4 ml

Conversion:	serotonin [ng/ml] x 5.67 = serotonin [nmol/l]
Content:	TRIS buffer with non-mercury preservatives, spiked with a defined quantity of serotonin

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)
- Reaction tubes, at least 3 ml, Polypropylene/Polystyrol

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 20 – 500 µl
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Vortex mixer

5. Sample collection, handling and storage

Serum
Collect blood by venipuncture, allow to clot, and separate serum by centrifugation according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time. Serum serotonin levels may fluctuate throughout the day. Therefore, the blood sample should always be taken at the same time of day. Traumatic vascular access can drastically increase serotonin levels.
When in doubt, it is recommended that hemolytic, icteric, and lipemic samples not be used in the assay (see 2.2.1).
Storage: up to 1 day at 18 – 25 °C; up to 3 days at 2 – 8 °C; storage for a longer period (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.
Always store samples protected from light.

Urine
24-hour urine samples as well as spontaneous urine (second morning urine) can be used for analysis.
24-hour urine: Over a defined period of 24 hours, all urine is collected in a bottle with acid (10 – 15 ml 100% acetic acid) provided for stabilization and the total volume is noted for evaluation of the results. During the collection period, the collected sample must always be stored in a cool place protected from light (2 – 8 °C).
Spontaneous urine (second morning urine): stabilized with 10 µl 100% acetic acid per 1 ml of urine sample can be used. Always store samples protected from light. A creatinine determination for normalization is required.
When stabilizing urine, consider the acidity (see 2.2.1).
Storage: up to 1 day at 18 – 25 °C; up to 3 days at 2 – 8 °C; storage for a longer period (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the reaction tubes and microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

⚠ *Do not exceed the temperature during the enzyme immunoassay of 20 – 25 °C and the prescribed incubation times. Too high temperature during the enzyme immunoassay and too long incubation times might influence the results.*

⚠ *Carry out the washing steps thoroughly! Poor washing might influence the results.*

6.1 Preparation of reagents and further notes

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50x** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Serotonin Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

Acylation Reagent
The Acylation Reagent (BA E-6612) has a freezing point of 18.5 °C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used.
If more than 3 ml are needed, pool the contents of the individual vials **ACYL-REAG** and mix thoroughly.

6.2 Preparation of samples – Acylation

1.	Pipette **20 µl** of the **standards, controls, and samples** into the respective reaction tubes.
2.	Add **500 µl** **ACYL-BUFF** to all tubes.
3.	Add **50 µl** of **ACYL-REAG** to all tubes.
4.	Mix the reaction tubes thoroughly (vortex) and incubate for **15 min** at **RT** (20 – 25 °C).
5.	Add **500 µl water** to all tubes and mix thoroughly (vortex).
⚠	Take **20 µl** of the **acylated standards**, **controls, and samples** for the **Serotonin ELISA**.

6.3 Serotonin ELISA

1.	Pipette **20 µl** of the **acylated standards, controls, and samples** into the appropriate wells of the **SER 5-HIAA**.
2.	Pipette **50 µl** of the **SER-AS** into all wells.
3.	Incubate **60 min at RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
4.	Discard or aspirate the content of the wells. Wash the plate **4 times** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **CONJUGATE** into each well.
6.	Incubate **for 30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
7.	Discard or aspirate the content of the wells. Wash the plate **4 times** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Pipette **100 µl** of the **SUBSTRATE** into each well.
9. ⚠	Incubate **for 25 ± 5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
10.	Add **100 µl** of the **STOP-SOLN** to all wells and shake the microtiter plate shortly.
11.	**Read** the absorbance of the solution in the wells within 10 min, using a microtiter plate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	Serotonin	
	Serum	8 – 2,170 ng/ml
	Urine	8 – 2,027 ng/ml

The standard curve, which can be used to determine the concentration of the unknown samples, is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

The concentrations of the samples and controls can be read directly from the standard curve.
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Standard A and must be re-assayed. For the calculation of the concentrations this dilution factor has to be taken into account.
The total amount of **Serotonin** excreted in urine during 24h is calculated as following:
µg/24h = µg/l x l/24h
The amount of **Serotonin** normalized to creatinine is calculated as following:
µg/g creatinine = ng/ml (serotonin) / creatinine (mg/dl) x 100

Conversion:
Serotonin [ng/ml] x 5.67 = serotonin [nmol/l]

7.1 Expected reference value

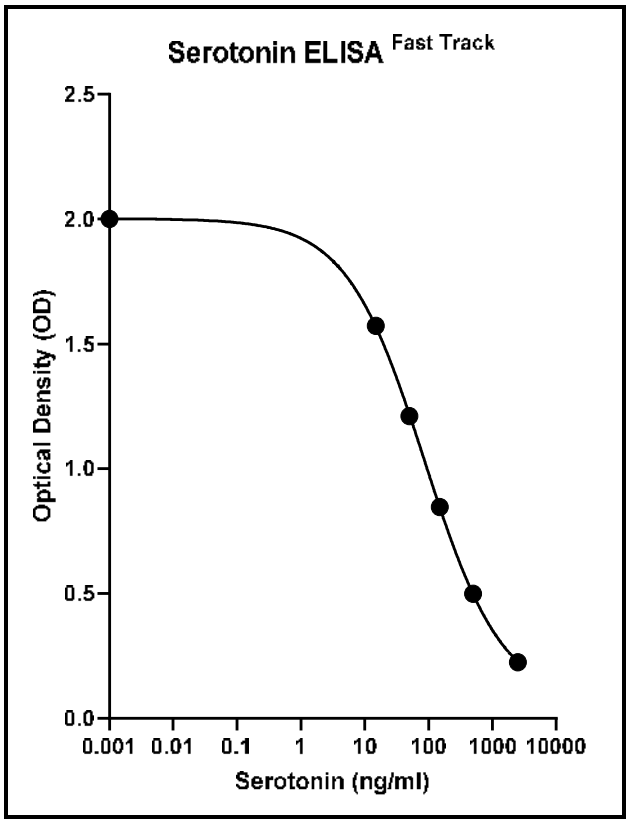
It is strongly recommended that each laboratory should determine its own reference values.
As a basis for the internal reference range determination, the following number of samples for the respective parameters was considered: 24-hour urine n = 194, spontaneous urine (second morning urine) n = 81, serum n = 80. Expected reference ranges were determined in an internal study by testing samples from an apparently healthy European population (95% reference interval).

	Serotonin
Reference range 24-hour urine	9 – 193 µg/24h 24 – 124 µg/g creatinine
Reference range Spontaneous urine (Second morning urine)	30 – 129 µg/g creatinine
Reference range serum	20 – 206 ng/ml

Values significantly outside the reference range should be assessed by a doctor.

7.2 Typical standard curve

⚠*Example: Do not use for calculation!*



8. Control samples

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. Commercially obtained control samples should be treated like unknown samples. Control samples should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

9. Assay characteristics

9.1 Performance data

Analytical sensitivity was determined according to the CLSI Standard EP17-A2 Vol. 32 No. 8.
For the determination of the analytical sensitivity, 5 blank samples and 5 low level samples in 2 kit lots in 4 replicates per sample were determined. This resulted in 60 results blank and 60 results low level per lot.

Analytical Sensitivity	**Serotonin**
Limit of Blank (LOB)	2.9 ng/ml
Limit of Detection (LOD)	5.9 ng/ml
Limit of Quantification (LOQ)	8.0 ng/ml

Analytical Specificity (Cross Reactivity)	
Substance	**Cross Reactivity (%)**
Tryptamine	0.171
Melatonin	< 0.1
5-Hydroxyindole acetic acid	< 0.1
Phenylalanine	< 0.1
Histidine	< 0.1
Tyramine	< 0.1
5-Hydroxytryptophan	< 0.1

The precision of the intra- and inter-assay variation was investigated by determining the concentration of 6 serum samples and 6 urine samples in two runs per day in each 2 replicates over 20 days (according to the CLSI Standard EP05-A3 Vol. 34 No.13).

Precision					
Intra-Assay			**Inter-Assay**		
Serum			**Serum**		
Sample	Mean ± SD [ng/ml]	CV [%]	Sample	Mean ± SD [ng/ml]	CV [%]
1	11.8 ± 2.1	17.6	1	11.8 ± 3.3	28.2
2	61.6 ± 5.2	8.4	2	61.6 ± 7.7	12.5
3	102 ± 8.6	8.5	3	102 ± 12.3	12.1
4	227 ± 15.5	6.8	4	227 ± 23.0	10.1
5	493 ± 25.2	5.1	5	493 ± 55.7	11.3
6	1,792 ± 109	6.1	6	1,792 ± 165	9.2
Urine			**Urine**		
Sample	Mean ± SD [ng/ml]	CV [%]	Sample	Mean ± SD [ng/ml]	CV [%]
1	18.1 ± 2.0	11.3	1	18.1 ± 4.0	22.2
2	55.2 ± 4.0	7.3	2	55.2 ± 6.4	11.7
3	153 ± 9.1	5.9	3	153 ± 14.6	9.5
4	240 ± 11.4	4.8	4	240 ± 21.9	9.1
5	498 ± 29.3	5.9	5	498 ± 44.5	8.9
6	1,798 ± 120	6.7	6	1,798 ± 221	12.3

Lot-to-Lot			
	Sample	Mean ± SD [ng/ml]	CV [%]
Serotonin in urine (n = 6)	1	103 ± 6.5	6.4
	2	734 ± 63.3	8.6
Serotonin in serum (n = 6)	1	97.6 ± 7.9	8.1
	2	790 ± 62.3	7.9

Recovery was determined according to the CLSI Standard EP 34 1st ed.

Recovery			
	Range [ng/ml]	Mean [%]	Range [%]
Serum	49.4 – 1,046	98	84 – 112
Urine	10.0 – 1,023	91	82 – 98

Linearity of sample dilution			
	Serial dilution up to	Mean [%]	Range [%]
Serum	1:64	103	93 – 113
Urine	1:64	98	88 – 111

The linearity within the measuring range was determined according to the CLSI Standard CLSI EP06-Ed2. The linearity is given if the determined value does not deviate by more than 20% from the forecast value.

Linear range	
Serum	18 – 2,170 ng/ml
Urine	20 – 2,027 ng/ml

The method comparison was conducted according to the CLSI Standard CLSI EP09c 3rd ed.

Method comparison ELISA vs. XLC-MS/MS	
Serum	$y = 0.99x - 9.2$; $r^2 = 0.996$; n = 100
Urine	$y = 0.9x - 20.7$; $r^2 = 0.988$; n = 97

9.2 Metrological Traceability

The values assigned to the standards and controls of the Serotonin ELISA Fast Track are traceable to SI Units by weighing with quality-controlled analyte.

Standards and Controls	Uncertainty [%]
	2.5

Serotonin ELISA Fast Track		
Serum	Concentration [ng/ml]	Expanded Uncertainty [%] k = 2*
	61.6	25.5
	227	20.8
Urine	Concentration [ng/ml]	Expanded Uncertainty [%] k = 2*
	18.1	44.7
	55.2	23.9
	153	19.6
	240	18.9
	498	18.5
	1798	25.1

* This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature

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For updated literature or any other information please contact your local supplier.

11. Changes

Version	Release Date	Chapter	Change
17.1	2022-06-14	All	- Assay Revision due to IVDR regulation (EU) 2017/746. Due to this revision all chapters were revised and changed.
18.0	2023-03-20	7.1	- Sentence: "Values significantly outside the reference range should be assessed by a doctor." added
19.0	2023-09-07	4.1 9.1	- Hazard labelling updated according to SDS - Lot-to-Lot updated
20.0	2025-04-25	2.1 4.1 7 9.2	- Updated - Hazard labelling updated according to SDS - Note added to the dilution factor in the calculation - Metrological Traceability updated

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE marking of conformity
	Caution		Catalogue number		Distributor
	Date of manufacture		Do not re-use		