DIAGNOSTIKA GMBH

Instructions for Use

Serotonin ELISA

Enzyme Immunoassay for the
Quantitative Determination of
Serotonin in Serum and Urine
and in EDTA-Plasma (RUO)

CE IVD

REF EA602/96

Σ 12 x 8

+2 +8 °C 2 – 8 °C

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Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of conformity
CONT	Content		Expiry Date
LOT	Lot Number	+2 +8 °C	Store at
	Manufactured by	Σ	Sufficient for ... determinations
REF	Catalogue Number	i	Consult Instructions for Use

1 Introduction and Principle of the Test

Serotonin (5-Hydroxytryptamine), a biogenic amine, is an intermediate product of the tryptophan metabolism. It is a well evaluated neurotransmitter of the central nervous system and can be found in high concentrations in the chromaffine cells of the intestinal mucosa, in the platelets and the serotonergic neurones of the brain.

Central-serotonergic neurones influence physiological functions such as sleep and the hormonal and cardio-vascular regulation. Increased serum levels can be found with malignant carcinoid, endogenous depression and schizophrenia. Serotonin is a specific tumor marker for the malignant carcinoid.

The assay kit provides materials for the quantitative measurement of derivated serotonin (5-Hydroxytryptamine) in human serum, plasma and urine. After sample preparation in the preparation plate, the derivatization takes place in the ELISA plate. In this process, serotonin is quantitatively converted into N-acylserotonin by the acylating reagent.

The Serotonin ELISA is a competitive enzyme immunoassay. Antigen bound to the solid phase and free antigen in solution compete for a defined number of antibody binding sites. When the system is in equilibrium, the unbound antigen-antibody complex is removed in a washing step and the correspondingly bound complex is detected using a peroxidase conjugate and determined via the conversion of tetramethylbenzidine (TMB). The TMB/POD reaction is stopped and measured at 450 nm. The concentration of the antigen-antibody complex bound to the solid phase is inversely proportional to the concentration of the antigen in the sample.

2 Precautions

- For in vitro diagnostic use only. For professional use only.
- Before carrying out the test, the valid instructions for use, as included in this kit, should be read completely and the content understood.
- Material of animal origin used in the preparation of the kit have been obtained from certified healthy animals but these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- When handling the reagents, controls and patient samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and safety goggles while performing the test.
- According to the CLP Regulation No. 1272/2008, the kit components do not have to be labelled as hazardous substances. Detailed safety information can be found in the safety data sheet.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents.
- Dispose of waste according to state and local environmental protection regulations.
- Broken glass can cause injury. Be cautious with glass vials.
- The quality control guidelines in the medical laboratory regarding the inclusion of control samples and/or pooled samples should be observed.
- One component contains a small amount of sodium azide as a preservative. Prevent the formation of heavy metal azides in the drain system by flushing copiously with water.

3 Storage and Stability

The kit is shipped at ambient temperature. Upon arrival, store the kit at 2 – 8 °C to keep it stable until its expiry date. Once opened the kit is stable until its expiry date. The shelf life of the ready-to-use reagents is indicated on the respective bottle label. For stability of prepared reagents refer to 6.1. Reagents must equilibrate to room temperature before use and refrigerated immediately after use.

4 Contents of the kit

MT-Strips **STRIPS** 12 strips

8 wells each, break apart, precoated with N-Acylserotonin

Standards 1 – 6 **CAL 1 - 6** 6 vials

each 4 ml, ready for use, Concentrations:

Standards	1	2	3	4	5	6
ng/ml	0	30	75	200	500	1500
nmol / l	0	170	426	1135	2838	8513

Control 1 & 2 **CON 1 & 2** 2 vials

Each 4 ml, ready for use

Range: see Q.C.-certificate

Acylation Buffer **ACYL-BUFF** 1 vial

35 ml, ready for use, blue coloured

Acylation Reagent **ACYL-REAG** 4 vials

3 ml, lyoph., dissolve content with 3 ml Solvent

Antiserum **AS** 1 vial

14 ml, ready for use, colour-coded yellow

Rabbit-anti-N-acylserotonin

Enzyme Conjugate **CONJ** 1 vial

14 ml, ready for use

Goat anti-rabbit-IgG-peroxidase

Wash Buffer **WASH** 1 vial

20 ml, concentrated (50x)

Substrate **SUB** 1 vial

14 ml, TMB solution, ready for use

Stop Solution **STOP** 1 vial
14 ml, ready for use,
contains 0.3 M sulphuric acid

Preparation Plate **PRE-PLATE** 1 piece
For sample preparation

Equalizing Reagent **EQUA-REAG** 1 vial
Lyoph., dissolve content with 35 ml Acylation Buffer

Solvent **SOLVENT** 1 vial
14 ml, ready for use, colour-coded yellow

Additional materials and equipment required, but not provided:

- Pipettes (10, 20, 50, 100 and 200 µl)
- Multipette
- Orbital shaker
- Multichannel pipette or microplate washing device
- Distilled water
- Vortex mixer and roller mixer
- Paper towels, pipette tips, timer
- Centrifuge
- Microplate photometer (450 nm)

5 Sample Collection

Serotonin is particularly sensitive to light; therefore the samples should be stored cooled and in the dark after collection.

Avoid repeated freezing and thawing of the samples.

Patients should avoid food and substances that affect the serotonin levels before drawing the blood sample or before and during the urine collection, including, for example, avocados, aubergines, pineapples, bananas, plums, mirabelle plums, currants, gooseberries, kiwis, melons, tomatoes, walnuts and coffee and substances such as methocarbamol, mephenesin, paractamol, salicylates, MAO inhibitors, nicotine.

5.1 Serum and Plasma

The test can be performed with serum as well as with EDTA plasma (the later for research use only). If plasma is to be used, special care must be taken to obtain true platelet-free plasma. Otherwise, the Serotonin level must be related to the number of thrombocytes in the sample. Since the preparation of platelet-free plasma requires special precautions, it is generally recommended to use serum instead of plasma.

Hemolytic, lipemic and icteric samples should not be used.

The samples can be stored up to 6 hours at 2 – 8 °C. For a longer storage (up to 6 months) the samples must be frozen at -20 °C

5.2 Urine

Both spontaneous urine and collected urine can be used.

Collected urine: The total volume of urine excreted during a 24-hours period should be collected and mixed in a single bottle containing 10 – 15 ml of 6 M hydrochloric acid (Warning: Observe hazard warnings) as preservative. Avoid exposure to direct sun light. Store in the dark during collection. Determine the total volume and take an aliquot for the measurement. For patients with suspected kidney disorders the creatinine concentration should be determined in addition. Urine samples can be stored at -20 °C for at least 6 months. Do not use non-acidified urine collection.

Mix and centrifuge urine before use.

6 Preparation of Reagents and Samples

Equilibrate reagents to room temperature

6.1 Preparation of Reagents

6.1.1 Wash Buffer

Dilute the content (20 ml) of 50x concentrated Wash Buffer WASH with dist. water to a total volume of 1,000 ml, mix briefly. For further use, the diluted wash buffer must be stored at 2 – 8 °C for a maximum period of 4 weeks.

Should the kit be used in several runs, then prepare only the required amount of wash buffer for each run.

6.1.2 Equalizing Reagent

Reconstitute the lyophilized Equalizing Reagent EQUA-REAG by transferring the complete content of the Acylation Buffer ACYL-BUFF into the vial. Vortex briefly and mix for at least 20 minutes on a roller mixer or similar shaker until completely dissolved. Thereby, avoid excessive formation of foam. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable until the expiry date.

6.1.3 Acylation Reagent

Remove the required amount of vials of Acylation Reagent ACYL-REAG from the foil pouch, leave the remaining vials inside together with the desiccant and close the pouch carefully. Reconstitute each vial of lyophilized Acylation Reagent with 3 mL of Solvent SOLVENT and mix on a roller mixer or similar shaker for at least 5 minutes. The Acylation Reagent should be freshly prepared immediately before the performing the test and is then stable for approx. 3 hours. The kit contains 4 vials of Acylation Reagent for multiple runs. When using the kit in one run, pool the dissolved contents of two vials. Discard the remaining reconstituted reagent after use.

All other reagents are ready for use.

6.2 Preparation of Samples

Duplicates are recommended. The wells of the Preparation Plate PRE-PLATE should be used only once. Please mark the respective wells before use.

1. **Pipette**
   10 µl Standards 1 – 6 CAL 1 – 6,
   10 µl Controls 1 & 2 CON 1 & 2,
   10 µl Serum,
   10 µl Urine,
   20 µl EDTA-Plasma into the respective wells of the Preparation Plate PRE-PLATE.
2. 200 µl Equalizing Reagent EQUA-REAG into each well.
3. Incubate for 5 minutes at room temperature on a orbital shaker at medium frequency.

Take 20 µl each for the ELISA.

7 Test Procedure

1. Pipette **20 µl each of diluted standards, controls and samples** into the respective wells of the coated microtiter strips [STRIPS]. Leave remaining microtiter strips in the foil pouch together with the desiccant and close carefully.
2. Pipette **50 µl Acylation Reagent** [ACYL-REAG] into each well and continue with point 3, immediately.
3. Incubate for 20 minutes at room temperature on an orbital shaker at medium frequency.
4. Pipette **100 µl Antiserum** [AS] into each well. Please use Multipette or similar (no single-channel or multi-channel pipettes).
5. Incubate for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium frequency.
6. Discard or aspirate the contents of the wells and wash thoroughly with **300 µl diluted Wash Buffer** [WASH] per well. Discard or aspirate the contents of the wells and remove residual liquid by tapping the inverted plate on a clean absorbent paper. Repeat the washing procedure 4 times. Alternatively, a washing device may be used.
7. Pipette **100 µl Enzyme Conjugate** [CONJ] into each well.
8. Incubate for 15 minutes at room temperature (20 – 25 °C) on an orbital shaker at medium frequency.
9. Wash: Repeat step 6.
10. Pipette **100 µl Substrate** [SUB] into each well.
11. Shake on an orbital shaker for 10 seconds and then incubate for 15 ± 5 minutes at room temperature, without shaking, on the table, cover plate with a large box.
12. Pipette **100 µl Stop Solution** [STOP] into each well, in the same order and timing as performed in step 10. Shake on an orbital shaker for 10 seconds.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer, within 15 minutes.

8 Calculation of the Results

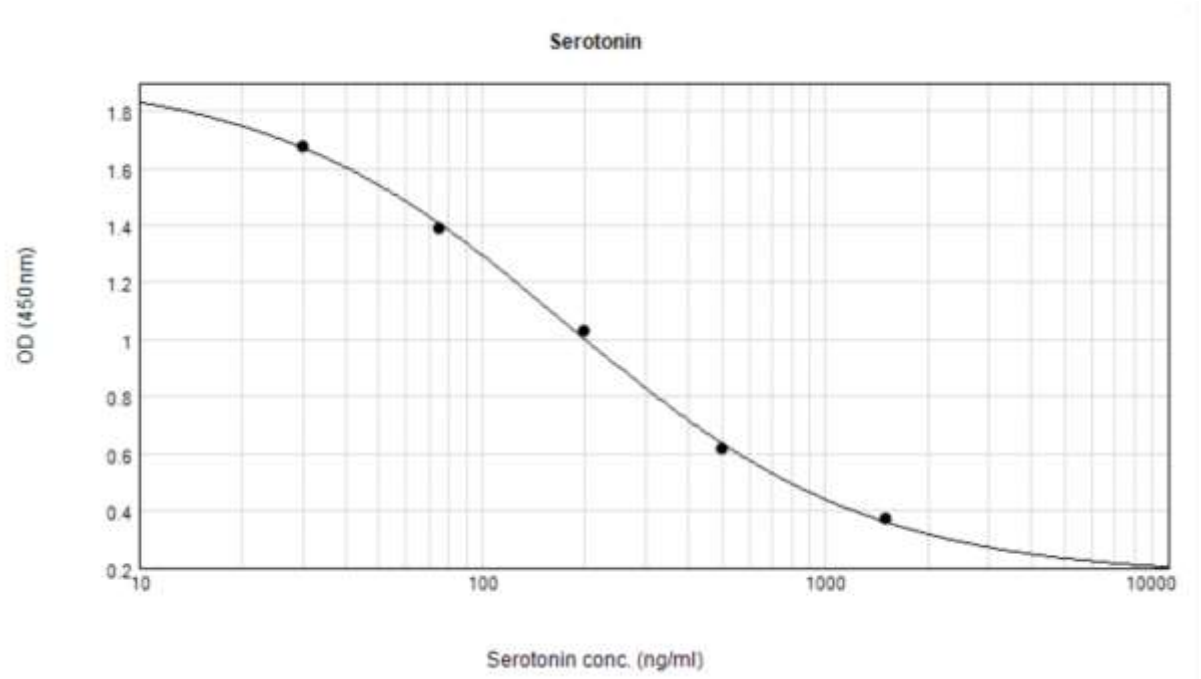
On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). When using analysing software, it is recommended to use the Four Parameter Logistic (4PL) Regression (alternatively: cubic-spline or logit-log).

Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max}, and then plotted on the y-axis. When using analysing software, it is recommended to use the Four Parameter Logistic (4PL) Regression (alternatively: cubic-spline or logit-log).

The concentration of the controls, urine and serum samples in ng/ml can be read directly from the standard curve.

The read-off values for plasma samples have to be divided by a factor of 2.

Typical standard curve:



Standard	1	2	3	4	5	6
ng/ml	0	30	75	200	500	1500
nmol/ l	0	170	426	1135	2838	8513

Conversion: Serotonin: 1ng/ml = 5.675 nmol/l

Quality Control: Test results are valid only if the kit controls are within the ranges specified on the QC Certificate. Otherwise, the test should be repeated.

9 Assay Characteristics

9.1 Normal Range

The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory establishes its own normal values.

Matrix	Reference range
Serum	40 - 200 ng/ml
Urine	< 250 µg/day

9.2 Sensitivity

Matrix	Lower Detection Limit	Calculation
Serum, Urine	11,8	OD_{Cal1} - 2xSD
Plasma	5,9	OD_{Cal1} - 2xSD

9.3 Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against Serotonin used in the ELISA method.

Substance	Cross Reactivity (%)
Serotonin	100
Tryptamine	1.9
5-Methoxytryptamine	0.37
Melatonin	< 0.019
5-Hydroxy-L-Tryptophan	< 0.019
5-HIAA	< 0.0019
L-Tryptophan	< 0.00174

9.4 Recovery

Increasing amounts of Serotonin were added to a serum, plasma and an urine sample. Each spiked sample was assayed. The analytical recovery of Serotonin was estimated at different concentrations by using the theoretically expected and the actually measured values.

Matrix	Range (ng/ml)	Mean (%)	Range (%)
Serum	112 - 720	104	98 - 109
Urine	48 - 681	105	93 - 116
Plasma	87 - 375	102	97 - 105

9.5 Linearity

The linearity of the ELISA method was investigated using different dilutions of a serum, plasma and an urine sample. Samples were diluted with distilled water.

Matrix	Range (ng/ml)	max. Dilution	Mean (%)	Range (%)
Serum	46 - 795	1 : 15	88	85 - 96
Urine	21 - 329	1 : 15	94	84 - 99
Plasma	29 - 333	1 : 10	88	84 - 93

9.6 Reproducibility

The reproducibility of the ELISA method was investigated by measuring the intra-assay-coefficients of variation (cv) by repeated measurements of different serum, plasma and urine samples with different Serotonin concentrations.

Matrix	Range (ng/ml)	Intra-Assay Variation cv (%)	Range (ng/ml)	Inter-Assay Variation cv (%)
Serum	121 – 649	7.4 - 6.6 %	110 – 591	7.7 – 6.3
Urine	115 – 429	7.9 – 4.1 %	139 – 399	8.7 – 6.1
Plasma	287	8.3 %	79 - 259	8.6 – 7.4

9.7 Comparison of Methods

Matrix	Method	Correlation
Serum	LC-MS	Y = 1.04 x LC-MS + 2; R = 0.981; N = 30
Urine	HPLC	Y = 0.91 x HPLC + 7; R = 0.972; N = 28

9.8 Calibration

The calibration is carried out by weighing the pure substance. The correctness of the method was determined by comparison of method (9.7).

9.9 Limitations of Method

The result of the Serotonin Elisa is to be seen in connection with other diagnostic procedures and the anamnesis and the resulting questions. Samples measured above the highest standard must be diluted with distilled water and reassayed. The values of diluted samples must be multiplied by the appropriate dilution factor.

9.10 Interferences

Hemolytic, lipemic and icteric specimens should not be used. Do not use non-acidified urine collection.

10 Changes to declare

Version _8: Note on sodium azide was added to Precautions (valid as of SER124).

Version _7: Extensive changes have been made and are highlighted in grey (valid as of SER123).

Version _6: IFU has been re-formatted. The manufacturer and distributor information have been changed. Component names as printed on labels were included in pipetting schemes to provide greater clarity. No changes have been made to components or execution of protocols.

11 Literature

- Kema, P.; de Vries, E.; Muskiet, F. (2000):
  Clinical chemistry of serotonin and metabolites
  Journal of Chromatography B, 747 33–48
- Lechin, F.; van der Dijs, B.; Lechin, A. (2005):
  Circulating Serotonin, Catecholamines, and Central Nervous System Circuitry Related to Some Cardiorespiratory, Vascular, and HematologicalDisorders
  The Journal of Applied Research Vol. 5, No. 4
- Spaeth, M. (2006):
  Fibromyalgia Syndrome: The Role of Neurochemicals
  Primary Psychiatry. 13(9):72-75
- Spivak, B.; Vered, Y.; Graff, E.;. et al. (1999):
  Low Platelet-Poor Plasma Concentrations of Serotoninin Patients with Combat-Related Posttraumatic Stress Disorder
  BIOL PSYCHIATRY. 45:840–845
- Ernberg, M.; Lundeberg, T.; Kopp, S. (2000):
  Pain and allodynia/hyperalgesia induced by intramuscular injection of serotonin in patients with fibromyalgia and healthy individuals
  Pain 85 31±39
- Alvarez, J.; Gluck, N.; Fallet, A.; et al. (1999):
  Plasma serotonin level after 1 day of fluoxetine treatment: a biological predictor for antidepressant response?
  Psychopharmacology 143: 97.101
- Mück-Seler, D.; Pivac, N.; Jakovljevic, M.; et al. (1999):
  Platelet Serotonin, Plasma Cortisol, and Dexamethasone Suppression Test in Schizophrenic Patients
  BIOL PSYCHIATRY 45:1433–1439
- Vikenes, K.; Farstad, M.; Nordrehaug, J. (1999):
  Serotonin Is Associated with Coronary Artery Disease and Cardiac Events
  Circulation August 3, 1999; 483-489
- Dayan, P.; Huys, Q.. (2008):
  Serotonin, Inhibition, and Negative Mood
  PLoS Computational Biology February 2008 | Volume 4 | Issue 2 | e4
- Leboyer, M.; Philippe, A.; Bouvard, M.; et al. (1999):
  Whole Blood Serotonin and Plasma Beta-Endorphin in Autistic Probands and Their First-Degree Relatives
  BIOL PSYCHIATRY 1999; 45:158–163

Pipetting Scheme

Sample Preparation

		Standards	Controls	Serum, Urine	EDTA-Plasma
PRE-PLATE:					
CAL 1 – 6	µl	10			
CON 1 & 2	µl		10		
Sample	µl			10	20
EQUA-REAG	µl	200	200	200	200

Shake plate for 5 minutes on an orbital shaker
Take 20 µl each for the ELISA

ELISA

		Diluted Standards	Diluted Controls	Diluted Serum, Urine	Diluted Plasma
STRIPS:					
Transfer from PRE-PLATE to STRIPS	µl	20	20	20	20
ACYL-REAG	µl	50	50	50	50

Immediately, incubate for 20 minutes at room temperature on an orbital shaker

AS	µl	100	100	100	100

Incubate for 30 minutes at room temperature on an orbital shaker
Wash 4 x with 300 µl WASH per well

CONJ	µl	100	100	100	100

Incubate for 15 minutes at room temperature on an orbital shaker
Wash 4 x with 300 µl WASH per well

SUB	µl	100	100	100	100

Shake plate for 10 seconds
Incubate for 15 ± 5 minutes at room temperature, without shaking.
Cover plate with large box.

STOP	µl	100	100	100	100

Shake plate for 10 seconds
Read absorbance at 450 nm