DIAGNOSTIKA GMBH

Instruction for use

Serotonin ELISA

Enzyme Immunoassay
for the Quantitative Determination of Serotonin
in Serum, Plasma and Urine

CE

IVD

REF EA602/96

Σ 12 x 8

+2 +8 °C 2 – 8 °C

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Contents

1. Introduction and Principle of the Test

Serotonin (5-Hydroxytryptamine), a biogenic amine, is a 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.

The assay kit provides materials for the quantitative measurement of derivated serotonin (5-Hydroxytryptamine) in serum, plasma and urine. The derivation is performed during the preparation of the samples. By using the acylation reagent the serotonin is quantitatively derivated into N-acylserotonin.

The competitive Serotonin ELISA kit uses the microtitre plate format. Serotonin is bound to the solid phase of the microtiter plate. Acylated serotonin and solid phase bound serotonin compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase serotonin is detected by anti-rabbit/peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase serotonin is inversely proportional to the serotonin concentration of the sample.

2. Precautions

- For in vitro use only.
- Disposable gloves and safety glasses should be used.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label.

3. Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
Do not use components beyond the expiration date shown on the kit labels.
Do not mix various lots of any kit component within an individual assay.

4. Contents of the Kit

4.1 **MT-Strips** **STRIPS** 12 strips
8 wells each, break apart
precoated with serotonin

4.2 **Standards 1 - 6** **CAL 1-6** 6 vials
Each 4 ml, ready for use
Concentrations:

Standard	1	2	3	4	5	6
ng/ml	0	15	50	150	500	2,500

4.3 **Control 1 & 2** **CONTROL 1 & 2** 2 vials
Each 4 ml, ready for use
Range: see q.c. certificate

4.4 **Acylation Buffer** **ACYL-BUFF** 1 vial
3 ml, ready for use, blue coloured

4.5 **Acylation Reagent** **ACYL-REAG** 1 vial
2.5 ml, ready for use

4.6 **Antiserum** **AS** 1 vial
11 ml, ready for use, colour coded yellow
Rabbit-anti-N-acylserotonin

4.7 **Enzyme Conjugate** **CONJ** 1 vial
12 ml, ready for use
Goat anti-rabbit-IgG-peroxidase

4.8 **Wash Buffer** **WASH** 1 vial
20 ml, 50x concentrated
Dilute contents with dist. water to 1 litre total volume.

4.9 **Substrate** **SUB** 1 vial
12 ml TMB solution, ready for use

4.10 **Stop Solution** **STOP** 1 vial
12 ml, ready for use
Contains 0.3 M sulphuric acid

4.11 **Reaction plate** **ACYL-PLATE** 1 piece
for acylation

4.12 **Equalizing Reagent** **EQUA-REAG** 1 vial
lyophilzed, dissolve content with 20.5 ml dist. water, dissolve carefully to minimize foam formation

Additional materials and equipment required but not provided:

- Pipettes (10, 25, 50, 100 and 200 µl)
- Orbital shaker
- Microplate washing device
- Microplate photometer (450 nm)

5. Sample Collection

5.1. Serum and Plasma

The test can be performed with serum as well as with EDTA plasma. If plasma is to be used care must be taken to get true platelet-free plasma. Otherwise, the Serotonin level has to 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 and lipemic 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

Repeated freezing and thawing should be avoided.

5.2. 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 as preservative. Avoid exposure to direct sun light. Determine the total volume and take an aliquot for the measurement. For patients with suspected kidney disorders the creatinine concentration should be tested, too. Urine samples can be stored at -20 °C for at least 6 months.

6. Preparation of Reagents and Samples

6.1. Microtiter strips **STRIPS**

Before opening the packet of strip wells, allow it to stand at room temperature for at least 10 minutes. After opening, keep any unused wells in the original foil packet with the desiccant provided. Reseal carefully and store at 2-8 °C.

6.2 Wash Buffer **WASH**

Dilute the content with dist. water to a total volume of 1,000 ml.
For further use the diluted wash buffer must be stored at 2 - 8 °C for a maximum period of 4 weeks.

6.3. Equalizing Reagent **EQUA-REAG**

Dissolve the content with 20.5 ml dist. water, mix shortly and leave on a roll mixer for 30 minutes. Handle carefully in order to minimize foam formation. The reconstituted Equalizing Reagent should be stored frozen at -20 °C and is stable for a minimum of 1 year.

All other reagents are ready for use.

6.4. Preparation of Samples (Acylation)

The wells of the reaction plate for the acylation can be used only once. Please mark the respective wells before using.

1. Pipette each 20 µl standard 1 - 6, each 20 µl control 1 & 2, each 20 µl serum, 20 µl urine or 40 µl plasma into the respective wells of the reaction plate.
2. Pipette each 20 µl Acylation Buffer into all wells.
3. Pipette each 200 µl Equalizing Reagent into all wells
   Shake plate on an orbital shaker for 10 seconds.
4. Pipette each 20 µl Acylation Reagent into all wells, mix immediately.
   Please note that Acylation Reagent reacts with many plastic materials including plastic trays. It does not react with normal pipette tips and with glass devices. Use an Eppendorf multipette or similar, fill the syringe directly from the vial and add well by well.
5. Incubate for 15 minutes at room temperature (approx. 20 °C) on an orbital shaker. Colour changed to green.
6. Take each 20 µl for the ELISA.

7. Test Procedure ELISA

7.1 Sample Incubation

Pipette each 20 µl prepared Standards 1 to 6, 20 µl prepared controls and 20 µl prepared samples into the respective wells of the coated microtiter strips (duplicates are recommended).

Pipette each 100 µl Antiserum into all wells.

Incubate for 30 minutes at room temperature on an orbital shaker.

7.2 Washing

Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer. Repeat the washing procedure 3 to 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

7.3 Conjugate Incubation

Pipette each 100 µl enzyme conjugate into all wells.
Incubate for 15 minutes at room temperature on an orbital shaker.

7.4 Washing

Repeat step 7.2.

7.5 Substrate Incubation

Pipette each 100 µl Substrate into all wells and incubate for 15 ± 5 minutes at room temperature on an orbital shaker.

7.6 Stopping

Pipette each 100 µl Stop Solution into all wells.

7.7 Reading

Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer.

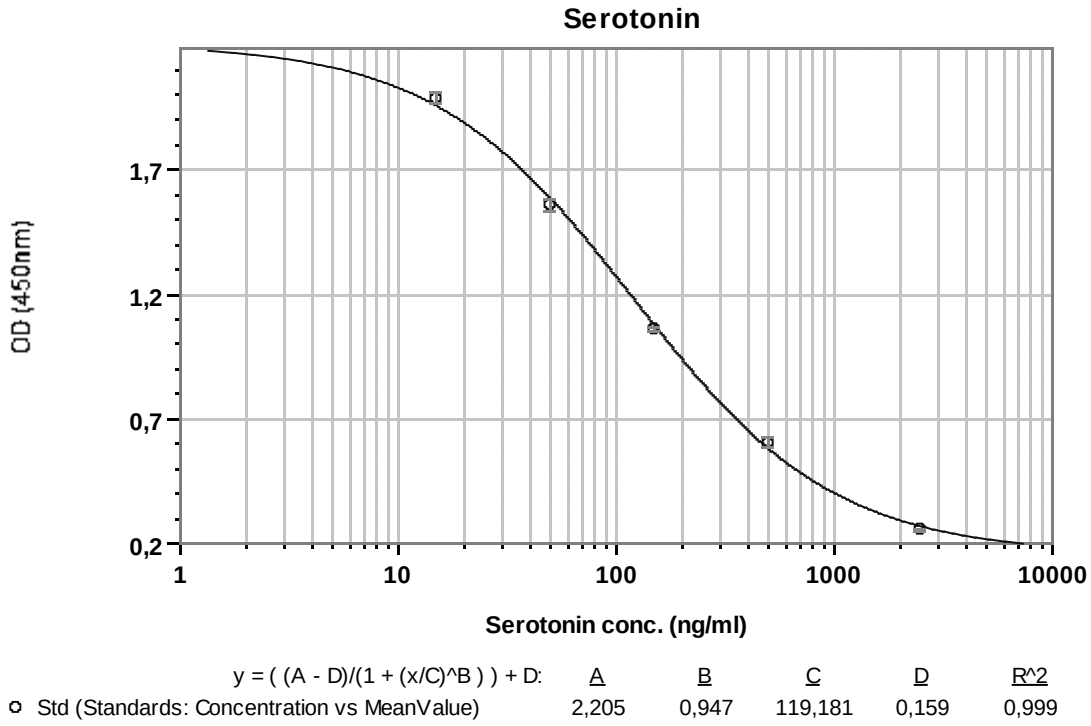
8. Calculation of the Results

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). 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.

The concentration of the controls, urine and serum samples can be read directly from this standard curve by using their average optical density.

The read-off values for plasma samples have to be divided by a factor of 1.8.

Typical standard curve:



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
○	Std (Standards: Concentration vs MeanValue)	2,205	0,947	119,181	0,159	0,999

Standard	1	2	3	4	5	6
ng/ml	0	15	50	150	500	2,500
nmol/ l	0	85.1	284	851	2,838	14,188

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

9. Assay Characteristics

Normal Range

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

Plasma (platelet-free): < 10 ng/ml

Serum:	Female	80 - 450 ng/ml
	Male	40 - 400 ng/ml
Urine:		50 - 250 µg/day

Sensitivity

4.7 ng/ml for serum and urine
2.6 ng/ml for plasma

Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against Serotonin used in the ELISA method. The tested compounds were Tryptamine, Melatonin, 5-HIAA, 5-Hydroxy-L-Tryptophan, 5-Methoxytryptamine and L-Tryptophan.

Substance	ED-50-Value (ng/ml)	Cross Reactivity (%)
Serotonin	133	**100**
Tryptamine	8,700	**1.5**
5-Methoxytryptamine	56,900	**0.23**
Melatonin	> 1,000,000	**< 0.0133**
5-Hydroxy-L-Tryptophan	> 1,000.000	**< 0.0133**
5-HIAA	> 10,000,000	**< 0.00133**
L-Tryptophan	> 10,000,000	**< 0.00133**

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	70 - 824	95	85 - 105
Urine	27 - 1085	105	83 - 120
Plasma	62 - 293	96	87 - 102

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	60 – 1,203	1 : 20	91	83 - 97
Urine	66 – 1,316	1 : 20	100	96 - 104
Plasma	79 - 395	1 : 5	94	90 - 97

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.

Intra-Assay Variation

Matrix	Range (ng/ml)	cv (%)
Serum	148 – 497	7.3 – 6.9
Urine	93 – 209	6.7 – 6.1
Plasma	163	7.7

10. Literature

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Pipetting Scheme

Sample Preparation

		Standards	Control	Serum, Urine	Plasma
Standard 1 - 6	µl	20			
Control 1 & 2	µl		20		
Serum, Urine	µl			20	
Plasma	µl				40
Acyl. Buffer	µl	20	20	20	20
Equalizing Reag.	µl	200	200	200	200

Shaking the plate for 10 seconds on an orbital shaker

Acyl. Reagent	µl	20	20	20	20

mix Immediately and incubate 15 minutes at room temperature on an orbital shaker

Take each 20 µl for the ELISA.

Pipetting Scheme ELISA

		Standard	Control	Sample
Standard 1 - 6	µl	20		
Control 1 & 2	µl		20	
Sample	µl			20
Antiserum	µl	100	100	100

30 minutes incubation at room temperature on an orbital shaker

3 - 4 x washing

Conjugate	µl	100	100	100

15 minutes incubation at room temperature on an orbital shaker

3 - 4 x washing

Substrate	µl	100	100	100

15 ± 5 minutes incubation at room temperature on an orbital shaker

Stop Solution	µl	100	100	100

Reading of absorbance at 450 nm