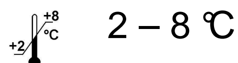
Instruction for use

Serotonin - ELISA

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

12 x 8



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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 th e 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
3.5 ml, ready for use

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

4.6 **Antiserum** | AS | 1 vial
5.5 ml, ready for use, colour coded blue
Rabbit-anti-N-acylserotonin

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

4.8 **Wash Buffer** | WASH 25x | 1 vial
20 ml, concentrated
Dilute content with dist. water to 500 ml 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 ml dist. water, dissolve carefully to minimize foam formation

4.13 **Adhesive Foil** FOIL 1 piece

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 and heparin 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 24 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 500 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 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. So please mark the respective wells before using.

1. Pipette each **10 µl standard 1 - 6**, each **10 µl control 1 & 2**, each **10 µl serum**, **25 µl plasma**, **10 µl urine** or **50 µl cerebrospinal fluid** into the respective wells of the reaction plate.
2. Pipette each **25 µl Acylation Buffer** into all wells.
3. Pipette each **200 µl Equalizing Reagent** into all wells.
4. Pipette each **25 µl Acylation Reagent** into all wells, mix **immediately.**
5. Incubate for 30 minutes at room temperature (approx. 20 °C) on an orbital shaker.
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 **50 µl Antiserum** into all wells.

Cover the plate with adhesive foil and incubate for 60 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 30 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 20 to 30 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.

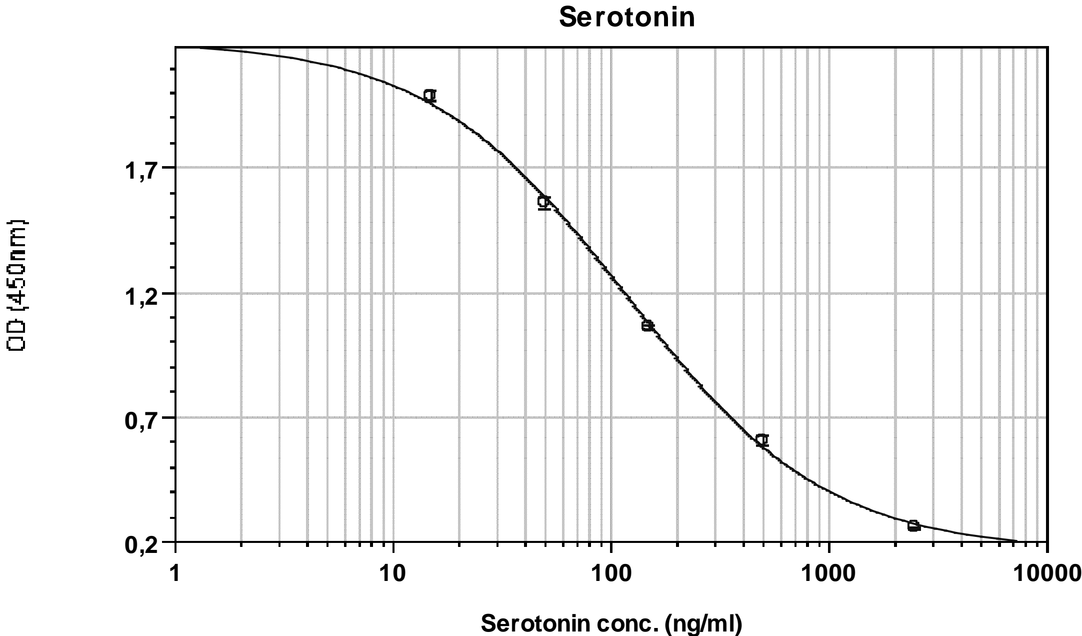
8. Calculation of the Results

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 2.4.
The values for cerebrospinal fluid samples have to be divided by a factor of 4.3.

Typical standard curve:



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

5 ng/ml

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-Methoxytryptamine and L-Tryptophan.

Substance	ED-50-Value (ng/ml)	Cross Reactivity (%)
Serotonin	131	**100**
Tryptamine	10,049	**1.3**
5-Methoxytryptamine	73,950	**0.18**
Melatonin	> 1,000,000	**< 0.013**
5-HIAA	> 1,000,000	**< 0.013**
L-Tryptophan	> 1,000,000	**< 0.013**

Recovery

Increasing amounts of Serotonin were added to a serum 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. The mean recoveries from all concentrations were for serum and urine 107%.
Concentrations in ng/ml

Serum:

added	measured	expected	% recovery
0	47.4		
19.6	65.3	67.0	97
38.5	94.4	85.9	110
68.4	134	116	116
96.8	160	144	111
125	205	172	119
152	220	199	110
188	235	235	100
244	322	291	111
310	418	357	117
385	456	432	105
476	481	523	92
684	729	731	100
874	932	921	101
	mean recovery		107

Urine:

added	measured	expected	% recovery
0	86.4		
19.6	99.7	106	94
38.5	137	125	110
68.4	174	155	112
96.8	200	183	109
125	208	211	98
152	261	238	109
188	294	274	107
244	370	330	112
310	406	396	102
385	516	471	109
476	645	562	115
684	845	770	110
874	985	960	103
	mean recovery		107

Linearity

The linearity of the ELISA method was investigated using different dilutions of a serum and an urine sample. The mean linearity from all dilutions were for serum and urine 103%.

Concentrations in ng/ml

Serum:

dilution	measured	recalculated value	recovery %
orig.	1077		
4 + 1	903	862	105
2 + 1	721	718	100
1 + 1	519	539	96
1 + 2	363	359	101
1 + 3	296	269	110
1 + 4	210	215	97
1 + 6	145	154	94
1 + 9	117	108	109
1 + 14	77.7	71.8	108
1 + 19	57.5	53.9	107

mean linearity 103

Urine:

dilution	measured	recalculated value	recovery %
orig.	1121		
4 + 1	842	897	94
2 + 1	812	747	109
1 + 1	585	561	104
1 + 2	391	374	105
1 + 3	277	280	99
1 + 4	196	224	87
1 + 6	165	160	103
1 + 9	123	112	110
1 + 14	89.7	74.7	120

mean linearity 103

Reproducibility

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

Intra-Assay Variation

Serum:

sample	n =	mean value	sd	cv (%)
1	41	133	8.0	6.0
2	41	366	17.4	4.7

Urine:

sample	n =	mean value	sd	cv (%)
1	40	128	8.4	6.6
2	40	308	16.6	5.4

10. Literature

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Pipetting Scheme

Sample Preparation

		Standards	Control	Serum, Urine	Plasma	Cerebr. Fluid
Standard 1 - 6	µl	10				
Control 1 & 2	µl		10			
Serum, Urine	µl			10		
Plasma	µl				25	
Cerebr. Fluid	µl					50
Acyl. Buffer	µl	25	25	25	25	25
Equalizing Reag.	µl	200	200	200	200	200
Acyl. Reagent	µl	25	25	25	25	25

30 minutes incubation at room temperature

Pipetting Scheme ELISA

		Standard	Control	Sample
Standard 1 - 6	µl	20		
Control 1 & 2	µl		20	
Sample	µl			20
Antiserum	µl	50	50	50

60 minutes incubation at room temperature

3 - 4 x washing

Enzyme Conjugate	µl	100	100	100

30 minutes incubation at room temperature

3 - 4 x washing

Substrate	µl	100	100	100

20 - 30 minutes incubation at room temperature

Stop Solution	µl	100	100	100

Reading of absorbance at 450 nm