



Serotonin ELISA ^{Fast Track}

40-734-350035

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Serotonin in serum, urine and platelets.

In the first step, Serotonin is quantitatively acylated.

The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum 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. The reaction is monitored at 450 nm.

Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of

the test kit for further instructions.

It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.

The appropriate safety data sheets of the individual products are available on the homepage. The

safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination

of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be

performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.

All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.

All reagents, however, should be treated as potential biohazards in use and for disposal.

3. **Storage and stability**

Store the reagents - **except of the Acylation Reagent (BA E-8912)** – at 2 - 8 °C until expiration date. The Acylation Reagent should be stored at room temperature. Do not use components beyond the expiry date indicated on the kit labels.

4.1 **Contents of the kit**

BA D-0023	REAC-TUBES	Reaction Tubes*)	2 x 50	ready for use
BA E-0030	WASH-CONC 50x	Wash Buffer Concentrate	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0045	CONJUGATE	Conjugate	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	Substrate	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	Stop Solution	1 x 12 mL	ready for use, containing 0.25 M H ₂ SO ₄
BA E-0931	96 SER 5-HIAA	Serotonin-5-HIAA Microtiter Strips	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated
BA R-8901	STANDARD A	Standard A	1 x 4 mL	ready for use
BA R-8902	STANDARD B	Standard B	1 x 4 mL	ready for use
BA R-8903	STANDARD C	Standard C	1 x 4 mL	ready for use
BA R-8904	STANDARD D	Standard D	1 x 4 mL	ready for use
BA R-8905	STANDARD E	Standard E	1 x 4 mL	ready for use
BA R-8906	STANDARD F	Standard F	1 x 4 mL	ready for use
BA E-8910	SER-AS	Serotonin Antiserum	1 x 12 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-8911	ACYL-BUFF	Acylation Buffer	1 x 55 mL	ready for use
BA E-8912	ACYL-REAG	Acylation Reagent	1 x 3 mL	ready for use
BA R-8951	CONTROL 1	Control 1	1 x 4 mL	ready for use
BA R-8952	CONTROL 2	Control 2	1 x 4 mL	ready for use

*) Instead of the reaction tubes it is also possible to use 48 wells microtiter plates for the sample preparation and acylation (please refer to 6.2). These plates are available upon request (BA D-0033).

4.2 **Additional materials and equipment required but not provided in the kit**

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

The assay can be performed with or without shaking. If a shaker is used, it should have the following characteristics: shaking amplitude 3mm; approx. 600 rpm.

5. **Sample collection and storage**

Repeated freezing and thawing of the samples should be avoided.

Serum

Haemolytic and especially lipemic samples should not be used with this assay.

Storage: up to 6 hours at 2 - 8°C; for longer periods (up to 6 months) at - 20°C.

Urine

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl can be used.

Storage: for longer periods (up to 6 months) at -20°C. Avoid exposure to direct sunlight.

Plasma

More than 98 percent of the circulating serotonin is located in the platelets and is released during blood clotting. Blood must be collected by venipuncture in plastic tubes containing EDTA or Citrate.

Platelet-rich plasma (PRP)

To obtain platelet-rich plasma (PRP) the samples are centrifuged for 10 minutes at room temperature (200 x g). Transfer the supernatant to another tube and count the platelets.

Platelets

The platelet pellet is obtained by adding 800 µL of physiological saline to 200 µL of PRP (containing between 350,000 – 500,000 platelets/µL) and centrifugation (4,500 x g, 10 minutes at 4 °C). The supernatant is then discarded.

200 µL of dist. water is added to the pellet and mixed thoroughly on a vortex mixer. This suspension can be stored frozen for several weeks at < -20°C.

After thawing of the frozen samples, centrifuge at 10,000 x g for 2 minutes at room temperature.

25 µL of the supernatant is used for the acylation reaction.

▲ For the determination of Serotonin in **platelet-free plasma** and **cerebrospinal fluid** the Serotonin Research™ ELISA (BA E-5900) should be used.

6. Test procedure for serum, urine and platelets

Allow all reagents and samples to reach room temperature. The measurement in duplicates is recommended.

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.

Storage: up to 6 months 2–8°C

Acylation Reagent

The Acylation Reagent (BA E-8912) 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. Alternative the Acylation Reagent can be stored at room temperature (20 – 25°C) separate from the other kit components (please refer to 3.).

6.2 Sample preparation and acylation of serum, urine and platelets

1.	Pipette 25 µL of standards , 25 µL of controls , and 25 µL of serum, urine or platelets into the respective Reaction Tubes .
2.	Add 500 µL of Acylation Buffer to all tubes.
3.	Add 25 µL of Acylation Reagent to all tubes.
4.	Mix thoroughly and incubate for 15 min at RT (20-25°C).
▲	Take 25 µL of the prepared standards, controls and samples for the Serotonin ELISA

6.3 Serotonin ELISA

The usage of a shaker is not mandatory. The alternative protocol without shaker is highlighted in italic and shaded in grey.

1.	Pipette 25 µL of the acylated standards, controls and samples into the appropriate wells of the Serotonin Microtiter Strips .
2.	Pipette 100 µL of the Serotonin Antiserum into all wells.
3.	Incubate 30 min at RT (20-25°C) on a shaker (approx. 600 rpm). <i>Without usage of a shaker: shake the Serotonin Microtiter Strips shortly by hand and incubate for 1 hour at RT (20-25°C).</i>
4.	Discard or aspirate the contents of the wells and wash each well 3 times thoroughly with 300 µL Wash buffer . Blot dry by tapping the inverted plate on absorbent material.
5.	Pipette 100 µL of the Conjugate into all wells.
6.	Incubate for 15 min at RT (20-25°C) on a shaker (approx. 600 rpm). <i>Without usage of a shaker: incubate for 15 min at RT (20-25°C).</i>
7.	Discard or aspirate the contents of the wells and wash each well 3 times thoroughly with 300 µL Washbuffer . Blot dry by tapping the inverted plate on absorbent material.
8.	Pipette 100 µL of the Substrate into all wells.
9.	Incubate for 15 ± 2 min at RT (20-25°C) on a shaker (approx. 600 rpm). <i>Without usage of a shaker: incubate for 15 min ± 2 min at RT (20-25°C).</i> Avoid exposure to direct sun light!

10. Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
11. **Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards					
Standard	A	B	C	D	E	F
Serotonin (ng/mL)	0	15	50	150	500	2 500
Serotonin (nmol/L)	0	85.1	284	851	2 840	14 175
Conversion:	Serotonin (ng/mL) x 5.67 = Serotonin (nmol/L)					

The calibration curve 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).

Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

The concentrations for **urine** and **serum samples** can be read directly from the calibration curve.

Calculation of serotonin in platelets

The content of serotonin in platelets is referred to 10^9 platelets.

Illustrative example:

Measured Serotonin concentration: 100 ng/mL

Number of the platelets in the PRP: $300\ 000 / \mu\text{L} = 0.3 \times 10^9$ platelets/mL with serotonin content of 100 ng.

The resulting serotonin content in the platelets is:

$333 \text{ ng} / 10^9 \text{ platelets}$ (100 ng serotonin x $1.0 \times 10^9 / 0.3 \times 10^9$)


7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

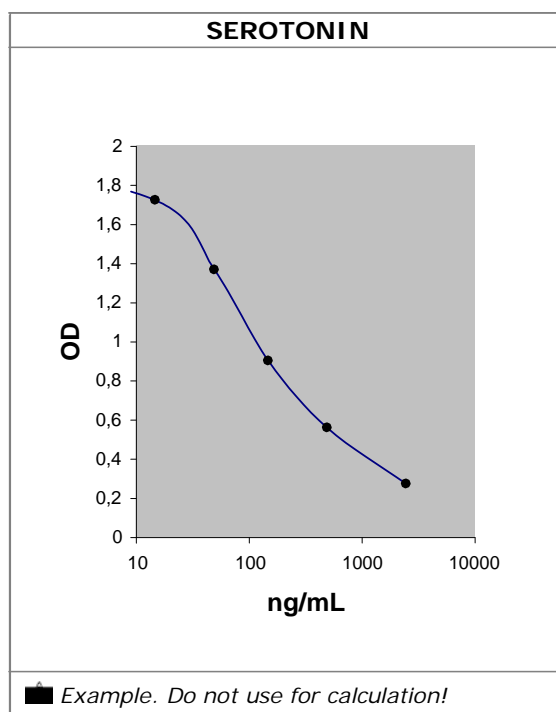
7.2 Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher

the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

 *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

7.3 Typical calibration curve:



8. Assay characteristics

Expected reference values			Serotonin				
	Serum		♀ 80 - 450 ng/mL	♂ 40 - 400 ng/mL			
	Urine		50 - 250 µg/day				
	Serotonin in platelets		215– 850 ng/10 ⁹ platelets				
Analytical Sensitivity (Limit of Detection)	Serotonin						
	Serum, urine and platelets						
				5 ng/mL			
Analytical Specificity (Cross Reactivity)	Substance		Cross Reactivity (%)				
			Serotonin				
	Serotonin		100				
	Tryptamine		0.19				
	Melatonin		0.03				
	5-Hydroxyindole acetic acid		<0.002				
	Phenylalanine		<0.002				
	Histidine		<0.002				
	Tyramine		<0.002				
5-Hydroxytryptophan		<0.002					
Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (ng/mL)	CV (%)		Sample	Range (ng/mL)	CV (%)
Serotonin	1	111 ± 7.3	6.6	Serotonin	1	84 ± 6.2	7.4
	2	355 ± 23	6.4		Urine	2	310 ± 25
Serotonin Serum	1	110 ± 6.0	5.4	Serotonin Serum	1	121 ± 7.4	6.0
	2	297 ± 12	3.9		2	333 ± 205	6.0
Linearity			Range	Serial dilution up to		Range (%)	
	Serotonin	Urine	40 - 860 ng/mL	1: 16		95 - 113	
		Serum	40 - 860 ng/mL	1: 16		92 -112	
Recovery			Mean (%)	Range (%)		% Recovery after spiking	
	Serotonin	Urine	94	86-102			
		Serum	97	87-102			

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= 0.99; n = 37

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