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Instructions for use

Serotonin ELISA

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

For *in-vitro* diagnostic use only.

REF

IB89546



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IVD

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Serotonin in serum, urine and platelets. For *in-vitro* diagnostic use only.

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.

1.2 Clinical application

Serotonin (5-hydroxytryptamine) is an intermediate product of tryptophan metabolism and is located primarily in the enterochromaffin cells of intestine (EC-cells), serotonergic neurons of the brain, platelets of the blood and is well established as a neurotransmitter in the central nervous system. EC-cell production accounts for 80% of the body's serotonin content. Serotonin is predominately metabolized to 5-hydroxyindoleacetic acid (5-HIAA), which is excreted by the kidneys.

Nearly all of the serotonin in circulating blood is concentrated in platelets. Altered concentrations of circulating serotonin have been implicated in several pathological conditions including chronic tension, headache, schizophrenia, hypertension, Huntington's disease, Duchenne's muscular dystrophy and early acute appendicitis.

The determination of serum serotonin levels is of high clinical significance for diagnostic assessment of carcinoid syndrome. An increasing interest in the determination of serotonin in platelets including uptake and release kinetics is expected in the near future.

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as under point "Procedural cautions, guidelines and warnings". 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.

The test result itself should never be the sole determinant for deriving any 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 has to 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) have to be followed.
- (4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
- (5) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- (6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- (7) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
- (8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- (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 ready 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 calibrator 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.

- (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) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- (16) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- (17) For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- (18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
- (19) 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.
- (20) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

Serum/Plasma

Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

24-hour urine

Please note the sample preparation! If the percentage of the final concentration of acid is too high, this will lead to incorrect results for the urine samples.

2.2.2 Drug interferences

Please refer to point "Sample collection and storage".

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA D-0023 **REAC-TUBES** **Reaction Tubes *)**- Ready to use

Contents: Reaction Tubes in a resealable pouch

Volume: 2 x 50 tubes

*) 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 (BA D-0033) are available upon request.

BA E-0030 **WASH-CONC 50x** **Wash Buffer Concentrate** - Concentrated 50x

Contents: Buffer with a non-ionic detergent and physiological pH

Volume: 1 x 20 ml/vial, light purple cap

BA E-0045 **CONJUGATE** **Enzyme Conjugate** - Ready to use

Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase

Volume: 1 x 12 ml/vial, red cap

BA E-0055 **SUBSTRATE** **Substrate** - Ready to use

Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide

Volume: 1 x 12 ml/vial, black cap

BA E-0080 **STOP-SOLN** **Stop Solution** - Ready to use

Contents: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, light grey cap



H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage

BA E-0931 **SER 5-HIAA** **Serotonin Microtiter Strips** - Ready to use

Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant

BA E-8910 **SER-AS** **Serotonin Antiserum** - Ready to use

Contents: Rabbit anti-serotonin antibody, blue colored

Volume: 1 x 12 ml/vial, blue cap

Standards and Controls - Ready to use

Cat. no.	Component	Color/Cap	Concentration ng/ml	Concentration nmol/l	Volume/ Vial
BA R-8901	STANDARD A	white	0	0	4 ml
BA R-8902	STANDARD B	light yellow	15	85.1	4 ml
BA R-8903	STANDARD C	orange	50	284	4 ml
BA R-8904	STANDARD D	dark blue	150	851	4 ml
BA R-8905	STANDARD E	light grey	500	2 840	4 ml
BA R-8906	STANDARD F	black	2 500	14 175	4 ml
BA R-8951	CONTROL 1	light green	Refer to vial labels for expected value and acceptable range!		4 ml
BA R-8952	CONTROL 2	dark red			4 ml

Conversion: Serotonin (ng/ml) x 5.67 = Serotonin (nmol/l)

Contents: TRIS buffer with non-mercury preservatives, spiked with defined quantity of serotonin

BA E-8912 **ACYL-REAG** **Acylation Reagent** - Ready to use

Contents: Acylation reagent in dimethylsulfoxide

Volume: 1 x 3 ml/vial, green cap

BA E-8911 **ACYL-BUFF** **Acylation Buffer** - Ready to use

Contents: TRIS buffer with non-mercury preservative

Volume: 1 x 55 ml/vial, light grey cap

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

- Calibrated precision pipettes to dispense volumes between 25 – 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
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

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

5. Sample collection and storage

Foods or liquids containing serotonin such as pineapple, eggplant, avocados, bananas, currants, kiwis, melon, mirabelles, plums, peaches chocolate, gooseberries, tomatoes, or walnuts, should be avoided 2 days before and including the day of the sample collection (24-hour urine). Selective Serotonin Reuptake Inhibitors (SSRIs) influence serotonin levels. People who are taking such medications should consult with their doctor before specimen collection.

Repeated freezing and thawing of the samples should be avoided.

Serum

Collect blood by venipuncture (monovette or vacuette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Haemolytic and especially lipemic samples should not be used for the assay.

Storage: up to 6 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.

Urine

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

Determine the total volume of urine excreted during a period of 24 h for calculation of the results.

Storage: up to 24 hours at 2-8°C, for longer periods (up to 6 months) at -20 °C.

Avoid exposure to direct sunlight.

Platelets

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 (monovette or vacuette) containing EDTA or Citrate as anticoagulant.

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.

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 water (deionized, distilled, or ultra-pure) 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-poor plasma** and **cerebrospinal fluid** the Serotonin Research™ ELISA (for details contact your local supplier) 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.

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.

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month at 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.

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.

6.2 Sample preparation and acylation of serum, urine and platelets

1.	Pipette 25 µl of standards, controls and 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 h at RT (20 – 25 °C).</i>
4.	Discard or aspirate the contents of the wells. Wash the plate 3 x 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 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. Wash the plate 3 x 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 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 sunlight!
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 (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	Serotonin
	10.2 – 2 500 ng/ml

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).

 *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 samples and have to be reported as being positive.*

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 10⁹ platelets.

Illustrative example:

Measured Serotonin concentration: 100 ng/ml

Number of the platelets in the PRP: 300 000 / µl = 0.3 x 10⁹ platelets/ml with serotonin content of 100 ng.

The resulting serotonin content in the platelets is:

333 ng/ 10⁹ platelets (100 ng serotonin x 1.0 x 10⁹/0.3 x 10⁹)

Conversion

Serotonin (ng/ml) x 5.67 = Serotonin (nmol/l)

Expected reference values

It is strongly recommended that each laboratory should determine its own reference values.

	Serotonin
Serum	70 – 270 ng/ml
Urine	50 - 250 µg/24h
Serotonin in platelets	500 - 950 ng/10 ⁹ platelets

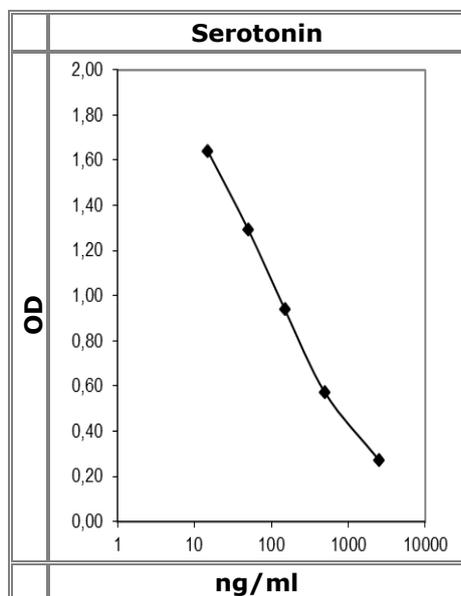
7.1 Quality control

It is recommended to use control samples according to national 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 Typical standard curve:



This example is a mean of 11 different runs; do not use for calculation!



8. Assay characteristics

Sensitivity	Limit of Detection (LOD)	6.2 ng/ml
	Limit of Quantitation (LOQ)	10.2 ng/ml

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
	Tryptamine	0.05
	Melatonin	0.08
	5-Hydroxyindole acetic acid	< 0.014
	Phenylalanine	< 0.014
	Histidine	< 0.019
	Tyramine	< 0.018
	5-Hydroxytryptophane	< 0.014

Precision							
Intra-Assay				Inter-Assay			
	Sample	Range (ng/ml) mean ± SD	CV (%)		Sample	Range (ng/ml) mean ± SD	CV (%)
Serotonin Urine (n = 40)	1	140.7 ± 16.3	11.6	Serotonin Urine (n = 15)	1	126.1 ± 14.2	11.3
	2	421.2 ± 38.6	9.2		2	414.5 ± 48.6	11.7
	3	1560 ± 215.3	13.8		3	1343 ± 200.2	14.9
Serotonin Serum (n = 20)	1	101.3 ± 9.6	9.7	Serotonin Serum (n = 7)	1	83.1 ± 10.3	12.4
	2	246.8 ± 31.2	12.6		2	244.3 ± 25.4	10.4
	3	667.5 ± 71.6	10.8				

Linearity			Range ng/ml	Serial dilution up to	Mean Linearity (%)	Range (%)
	Serotonin	Urine	30 - 3500	1:65	100	88 - 118
		Serum	40 - 3000	1:33	96	80 - 113

Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Serotonin	Urine	96	74 - 105	
		Serum	108	89 - 126	

Method comparison versus RIA*	Urine Serum	$y = 0,94x + 19.58; R^2 = 0,98$ $y = 0,85x + 33.18; R^2 = 0,97$
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*Commercial available RIA

9. References/Literature

- (1) Oliveira et al. Disturbances of Wnt/ β -catenin pathway and energy metabolism in early CKD: effect of phosphate binders. Nephrol Dial Transplant, 28(10):2510-2517 (2013)
- (2) Shahin et al. Detection of Plasma and Urinary Monoamines and Their Metabolites in Nonsegmental Vitiligo. Acta Dermatovenerol Croat, 20(1):14-20 (2012)
- (3) Ciprandi et al. Serotonin in Allergic Rhinitis: a Possible Role for Behavioural Symptoms, 10(3):183-188 (2011)

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

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!