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Instructions for use

Serotonin ELISA Fast Track

REF BA E-8900R

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

1. Introduction

1.1 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.

1.2 Background

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.

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) The principles of Good Laboratory Practice (GLP) have to be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(4) 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.
(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(6) 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.
(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(8) 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.
(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(11) A calibrator curve must be established for each run.
(12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(13) 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.
(14) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(15) 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.
(16) For information on hazardous substances included in the kit please refer to Safety Data Sheets (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(17) 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 might cause inaccurate results.
Hemolytic samples (up to 4 mg/ml hemoglobin), icteric samples (up to 50 mg/dl bilirubin) and lipemic samples (up to 1700 mg/dl triglycerides) have no influence on the assay 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 macrotiter 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
Hazards identification:

H290 May be corrosive to metals.

BA E-0931 IVD 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 coloured
Volume: 1 x 12 ml/vial, blue cap

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

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour / 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

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 according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred.
Haemolytic, icteric and 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 venepuncture according to manufacturer's instructions 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. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

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). ***Without usage of a shaker:*** *shake the* ***Serotonin Microtiter Strips*** *shortly by hand and incubate for* ***1 h*** *at* ***RT*** *(20 – 25 °C).*
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). ***Without usage of a shaker:*** *incubate for* ***15 min at RT*** *(20 – 25 °C).*
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).
⚠	***Without usage of a shaker:*** *incubate for* ***15 min ± 2 min*** *at* ***RT*** *(20 – 25 °C).* ***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 sample 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 10^9 platelets/ml with serotonin content of 100 ng.
The resulting serotonin content in the platelets is:
333 ng/ 10^9 platelets (100 ng serotonin x 1.0 x 10^9 /0.3 x 10^9)

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.

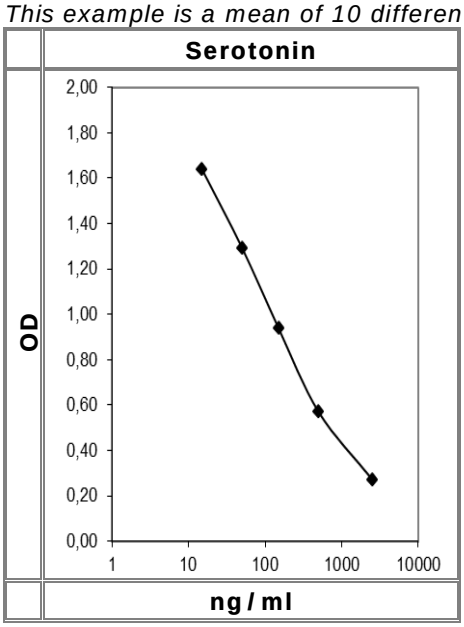
	Serotonin
Serum	70 – 270 ng/ml
24-hour urine	50 - 250 µg/24h
Serotonin in platelets	500 - 950 ng/10^9 platelets

7.1 Quality control

The confidence limits of the kit controls are printed on the QC-Report.

7.2 Typical standard curve:

⚠ *This example is a mean of 10 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

	Substance	**Cross Reactivity (%)**
Analytical Specificity (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 (%)
	Urine	30 - 3500	1:65	100	88 - 118
	Serum	40 - 3000	1:33	96	80 -113

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

Method comparison versus RIA*	Urine	$y = 0.94x + 19.58$; $R^2 = 0.98$
	Serum	$y = 0.85x + 33.18$; $R^2 = 0.97$

***Commercial available RIA

9. References/Literature

(1) Oliveira et al. Disturbances of W nt/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:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!