



User's Manual

Serotonin ELISA kit

REF DEIA-JY2408

 96T

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Enzyme Immunoassay for the quantitative determination of serotonin in urine and serum, to evaluate serotonin homeostasis. The determination of serotonin in urine is helpful for the determination of neurostress.

General Description

Serotonin (5-hydroxytryptamine) is an intermediate product of tryptophan metabolism, a wellstudied neurotransmitter, and may also act as a peripheral hormone. Synthesis occurs mainly in enterochromaffin cells (ec-cells) of the gastrointestinal tract and in neurons. It is present in high concentrations in ec-cells of the intestine, serotonergic neurons of the brain, and platelets. Serotonin is mainly degraded to 5-hydroxyindole acetic acid (5-HIAA) or melatonin and can be excreted in the urine. In the bloodstream, the vast majority of serotonin is found in platelets and can be readily detected in serum. Altered serotonin levels in serum and/or urine can indicate both physical and psychological dysfunction. Serotonin balance may be impaired in serum and/or urine in a variety of conditions. For example, decreased serotonin levels have been demonstrated in depression, anxiety, and even pain sensitivity compared to unaffected subjects. Increased serotonin levels, on the other hand, have been reported in patients with serotonin-secreting neuroendocrine tumors, also called carcinoid tumors, or hepatocellular carcinomas.

Principles of Testing

The quantitative determination of serotonin follows the basic principles of the enzyme immunoassay. 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. Manual processing is recommended. The use of automatic laboratory equipment is the responsibility of the user.

Reagents And Materials Provided

1. **WASH-CONC 50x Wash Buffer Concentrate** - concentrated 50x

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

Volume: 1 x 20 ml/vial, purple cap

2. **CONJUGATE Antibody Conjugate** – ready to use

Content: Goat anti-rabbit immunoglobulins conjugated with peroxidase

Volume: 1 x 12 ml/vial

Description: Species is goat, red cap

3. **SUBSTRATE Substrate** – ready to use

Content: Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide

Volume: 1 x 12 ml/vial, black cap

4. **STOP-SOLN Stop Solution** – ready to use

Content: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, grey cap

5. **LI SER 5-HIAA Serotonin Microtiter Strips** – ready to use

Content: 1 x 96 well (12x8) antibody precoated microwell plate in a resealable white pouch with desiccant

6. **ACYL-REAG Acylation Reagent** – ready to use

Content: Acylation reagent in DMSO

Volume: 2 x 3 ml/vial, white cap

7. **SER-AS Serotonin Antiserum** – ready to use

Content: Rabbit anti-Serotonin antibody, blue coloured

Volume: 1 x 6 ml/vial, blue cap

Description: Species is rabbit

8. **ACYL-BUFF Acylation Buffer** – ready to use

Content: TRIS buffer with non-mercury preservative

Volume: 1 x 55 ml/vial, grey cap

9. **Calibration and Controls**

Standards and Controls – ready to use

STANDARD A white 0 ng/ml, 0 nmol/l. 4 ml

STANDARD B yellow 15 ng/ml, 85 nmol/l. 4 ml

STANDARD C orange 50 ng/ml, 284 nmol/l. 4 ml

STANDARD D blue 150 ng/ml, 851 nmol/l. 4 ml

STANDARD E grey 500 ng/ml, ng/ml, 2,840 nmol/l. 4 ml

STANDARD F black 2,500 ng/ml, 14,175 nmol/l. 4 ml

CONTROL 1 green Refer to COA for expected value and acceptable range! 4 ml

CONTROL 2 red Refer to COA for expected value and acceptable range! 4 ml

Conversion: serotonin [ng/ml] x 5.67 = serotonin [nmol/l]

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

Materials Required But Not Supplied

1. Water (deionized, distilled, or ultra-pure)
2. Absorbent material (paper towel)
3. Reaction tubes, at least 3 ml, Polypropylene/Polystyrol

4. Calibrated precision pipettes to dispense volumes between 20 – 500 µl
5. Microtiter plate washing device (manual, semi-automated or automated)
6. ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
7. Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
8. Vortex mixer

Storage

Store kit and 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 2 months when stored at 2 – 8°C. Once the resealable pouch has been opened, care should be taken to close it tightly again including the desiccant.

Specimen Collection And Preparation

Serum

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time. Serum serotonin levels may fluctuate throughout the day. Therefore, the blood sample should always be taken at the same time of day. Traumatic vascular access can drastically increase serotonin levels.

When in doubt, it is recommended that hemolytic, icteric, and lipemic samples not be used in the assay.

Storage: up to 1 day at 18 – 25°C; up to 3 days at 2 – 8°C; storage for a longer period (up to 6 months) at -20°C.

Repeated freezing and thawing should be avoided.

Always store samples protected from light.

Urine

24- hour urine samples as well as spontaneous urine (second morning urine) can be used for analysis. 24-hour urine: Over a defined period of 24 hours, all urine is collected in a bottle with acid (10 – 15 ml 100% acetic acid) provided for stabilization and the total volume is noted for evaluation of the results.

During the collection period, the collected sample must always be stored in a cool place protected from light (2 – 8°C).

Spontaneous urine (second morning urine): stabilized with 10 µl 100% acetic acid per 1 ml of urine sample can be used. Always store samples protected from light. A creatinine determination for normalization is required.

Storage: up to 1 day at 18 – 25°C; up to 3 days at 2 – 8°C; storage for a longer period (up to 6 months) at -20°C.

Repeated freezing and thawing should be avoided.

Reagent Preparation

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate WASH-CONC 50x with water to a final volume of 1000 ml.

Storage: 2 months at 2 – 8 °C

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.

Acylation Reagent

The Acylation Reagent 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.

If more than 3 ml are needed, pool the contents of the individual vials ACYL-REAG and mix thoroughly.

Preparation of samples – Acylation

1. Pipette 20 µl of the standards, controls, and samples into the respective reaction tubes.
2. Add 500 µl ACYL-BUFF to all tubes.
3. Add 50 µl of ACYL-REAG to all tubes.
4. Mix the reaction tubes thoroughly (vortex) and incubate for 15 min at RT (20 – 25 °C).
5. Add 500 µl water to all tubes and mix thoroughly (vortex).

Note: Take 20 µl of the acylated standards, controls, and samples for the Serotonin ELISA.

Assay Procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the reaction tubes and microwell plates (microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.


The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.

Note: The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.

Note: Do not exceed the temperature during the enzyme immunoassay of 20 – 25 °C and the prescribed incubation times. Too high temperature during the enzyme immunoassay and too long incubation times might influence the results.

Note: Carry out the washing steps thoroughly! Poor washing might influence the results.

Serotonin ELISA

1. Pipette 20 µl of the acylated standards, controls, and samples into the appropriate wells of the  SER 5-HIAA.
2. Pipette 50 µl of the SER-AS into all wells.
3. Incubate 60 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
4. Discard or aspirate the content of the wells. Wash the plate 4 times 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 each well.
6. Incubate for 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
7. Discard or aspirate the content of the wells. Wash the plate 4 times 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 each well.
9. Incubate for 25 ± 5 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm). Avoid exposure to direct sunlight!
10. Add 100 µl of the STOP-SOLN to all wells and shake the microtiter plate shortly.
11. Read the absorbance of the solution in the wells within 10 min, using a microtiter plate reader set to 450 nm (if available a reference wavelength between 620 nm and 650 nm is recommended).

Quality Control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. Commercially obtained control samples should be treated like unknown samples.

Control samples should fall within established confidence limits. The confidence limits of the kit controls are printed on the COA.

Calculation

Measuring range

Serum 8 – 2,170 ng/ml

Urine 8 – 2,027 ng/ml

The standard 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) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e. g., 4-parameter, marquardt).

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 must be reported as being positive.

The concentrations of the samples and controls can be read directly from the standard curve.

Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with Standard A and must be re-assayed.

The total amount of Serotonin excreted in urine during 24h is calculated as following:

$$\mu\text{g}/24\text{h} = \mu\text{g}/\text{l} \times \text{l}/24\text{h}$$

The amount of Serotonin normalized to creatinine is calculated as following:

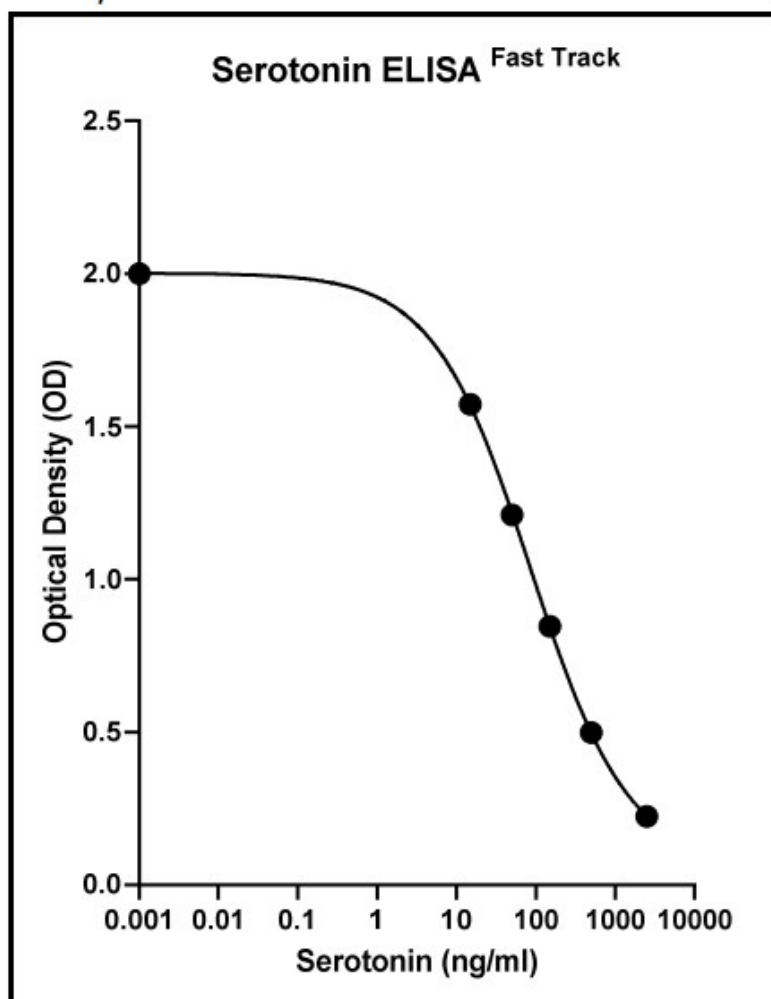
$$\mu\text{g}/\text{g creatinine} = \text{ng}/\text{ml (serotonin)} / \text{creatinine (mg}/\text{dl)} \times 100$$

Conversion

$$\text{Serotonin [ng/ml]} \times 5.67 = \text{serotonin [nmol/l]}$$

Typical Standard Curve

Example do not use for calculation!



Reference Values

It is strongly recommended that each laboratory should determine its own reference values. As a basis for the internal reference range determination, the following number of samples for the respective parameters was considered: 24-hour urine $n = 194$, spontaneous urine (second morning urine) $n = 81$, serum $n = 80$. Expected reference ranges were determined in an internal study by testing samples from an apparently healthy European population (95% reference interval).

Reference range: 9 – 193 $\mu\text{g}/24\text{h}$

24- hour urine: 24 – 124 $\mu\text{g}/\text{g}$ creatinine

Reference range - Spontaneous urine (Second morning urine): 30 – 129 $\mu\text{g}/\text{g}$ creatinine

Reference range - Serum: 20 – 206 ng/ml

Performance Characteristics

Analytical Sensitivity was determined according to the CLSI Standard EP17-A2 Vol. 32 No. 8.

For the determination of the analytical sensitivity, 5 blank samples and 5 low level samples in 2 kit lots in 4 replicates per sample were determined. This resulted in 60 results blank and 60 results low level per lot.

Analytical Sensitivity	Serotonin
Limit of Blank (LOB)	2.9 ng/ml
Limit of Detection (LOD)	5.9 ng/ml
Limit of Quantification (LOQ)	8.0 ng/ml

Analytical Specificity (Cross Reactivity)	
Substance	Cross Reactivity (%)
Tryptamine	0.171
Melatonin	< 0.1
5-Hydroxyindole acetic acid	< 0.1
Phenylalanine	< 0.1
Histidine	< 0.1
Tyramine	< 0.1
5-Hydroxytryptophan	< 0.1

The method comparison was conducted according to the CLSI standard CLSI EP09c 3rd ed.

Method comparison ELISA vs. XLC-MS/MS	Serum	$y = 0.99x - 9.2; r^2 = 0.996; n = 100$
	Urine	$y = 0.9x - 20.7; r^2 = 0.988; n = 97$

The precision of the intra- and inter-assay variation was investigated by determining the concentration of 6 serum samples and 6 urine samples in two runs per day in each 2 replicates over 20 days (according to the CLSI standard EP05-A3 Vol. 34 No.13).

Specificity

NA

Linearity

The linearity within the measuring range was determined according to the CLSI standard CLSI EP06- Ed2. The linearity is given if the determined value does not deviate by more than 20% from the forecast value.

Recovery			
	Range [ng/ml]	Mean [%]	Range [%]
Serum	49.4 – 1,046	98	84 – 112
Urine	10.0 – 1,023	91	82 – 98

Linearity of sample dilution			
	Serial dilution up to	Mean [%]	Range [%]
Serum	1:64	103	93 – 113
Urine	1:64	98	88 – 111

Linear range

Serum 18 – 2,170 ng/ml

Urine 20 – 2,027 ng/ml

Recovery

Recovery was determined according to the CLSI standard EP 34 1st ed.

Precision					
Intra-Assay			Inter-Assay		
Serum			Serum		
Sample	Mean \pm SD [ng/ml]	CV [%]	Sample	Mean \pm SD [ng/ml]	CV [%]
1	11.8 \pm 2.1	17.6	1	11.8 \pm 3.3	28.2
2	61.6 \pm 5.2	8.4	2	61.6 \pm 7.7	12.5
3	102 \pm 8.6	8.5	3	102 \pm 12.3	12.1
4	227 \pm 15.5	6.8	4	227 \pm 23.0	10.1
5	493 \pm 25.2	5.1	5	493 \pm 55.7	11.3
6	1,792 \pm 109	6.1	6	1,792 \pm 165	9.2

Precautions

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 must 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. 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 must be followed.
4. To reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
5. If serious incidents should occur in connection with this product, they should be reported to the manufacturer and the competent national authorities.
6. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultrapure water. Avoid repeated freezing and thawing of reagents and specimens.
7. The microplate contains snap-off strips. Unused wells must be stored at 2 – 8°C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
8. Duplicate determination of sample is highly recommended.
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 for use 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 standard 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 COA provided with the kit.
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.
17. The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
18. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
19. The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
20. In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.
21. The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but must be correlated to other diagnostic tests and clinical observations.

Limitations

Interfering substances and proper handling of specimens

Urine

Please note the sample collection! It cannot be excluded that high acid concentrations lead to incorrect results. Up to 30 µl 100% acetic acid per 1 ml urine no influence on the results was observed.

Serum

Samples containing precipitates or fibrin strands might cause inaccurate results. Hemolytic samples (up to 2 mg/ml hemoglobin), icteric samples (up to 50 mg/dl bilirubin) and lipemic samples (up to 834 mg/dl triglycerides) have no influence on the assay results. If the concentrations cannot be estimated and there are doubts as to whether the above limit values for hemolytic, icteric or lipemic samples are complied with, the samples should not be used in the assay.

Drug and food interferences

The following foods and stimulants can affect the serotonin content in the sample. Alcohol, pineapple, eggplant, avocados, bananas, grapefruit, currants, cocoa, kiwis, caffeine, melons, mirabelles, nicotine, pecans, peaches, plums, chocolate, gooseberries, tomatoes, walnuts. Some drugs can also affect serotonin levels in the sample. For example, taking amphetamines, acetanilide, coumarins, ephedrine, guaifenesin, mephensin (carbamate), methocarbamol, monoamine oxidase inhibitors (MAO inhibitors), acetaminophen, phenacetin, phenobarbital, phentolamine, or reserpine can lead to increased serotonin levels. In contrast, acetylsalicylic acid, chlorpromazine, isoniazid, levodopa, methenamine, methyl dopa, promethazine, selective serotonin reuptake inhibitors (SSRIs), or streptozocin may result in decreased serotonin levels.

Therefore, 2 – 4 days prior to specimen collection, these foods should be avoided and the medications discontinued if medically justifiable.

High-Dose-Hook effect

No hook effect was observed in this test.