Serotonin ELISA Kit

Catalog Number KA1894

96 assays

Version: 14

Intended for research use only

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Introduction

Intended Use

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

Background

Serotonin (5-hydroxytryptamine) is an intermediate product of tryptophan metabolism [1], a well-studied neurotransmitter, and may also act as a peripheral hormone [2]. Synthesis occurs mainly in enterochromaffin cells (ec-cells) of the gastrointestinal tract and in neurons [1, 3]. It is present in high concentrations in ec-cells of the intestine, serotonergic neurons of the brain, and platelets [1, 3 – 6]. Serotonin is mainly degraded to 5-hydroxyindole acetic acid (5-HIAA) or melatonin [1, 7] and can be excreted in the urine [8]. In the bloodstream, the vast majority of serotonin is found in platelets [9] and can be readily detected in serum.

Principle of the Assay

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.
This product is not intended to clinical diagnoses.

General Information

Materials Supplied

List of component

Component	Amount
Wash Buffer Concentrate (50x): Buffer with a non-ionic detergent and physiology pH.	20 mL
Conjugate: Ready to use, goat anti-rabbit immunoglobulins conjugated with peroxidase.	12 mL
Substrate: Ready to use, chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide.	12 mL
Stop Solution: Ready for use, containing 0.25 M sulfuric acid. Hazards identification:  H290 May be corrosive to metals.	12 mL
Serotonin Microtiter Strips: Ready to use, antibody precoated microwell plate in a resealable white pouch with desiccant.	96 (8x12) wells
Serotonin Antiserum: Ready to use, rabbit anti-serotonin antibody, blue coloured.	6 mL
Acylation Reagent: Acylation reagent in DMSO, ready to use.	2 x 3 mL
Acylation Buffer: Tris buffer with non-mercury preservative, ready to use.	55 mL

Standards and Controls - Ready to use

Component	Colour/Cap	Concentration (ng/mL)	Concentration (nmol/L)	Amount
Standard A	white	0	0	4 mL
Standard B	yellow	15	85	4 mL
Standard C	orange	50	284	4 mL
Standard D	blue	150	851	4 mL
Standard E	grey	500	2840	4 mL
Standard F	black	2500	14175	4 mL
Control 1	green	Refer to vial labels for expected value and acceptable range.		4 mL
Control 2	red	Refer to vial labels for expected value and acceptable range.		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

Storage Instruction

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 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.

Materials Required but Not Supplied

- ✓ Calibrated precision pipettes to dispense volumes between 20-500 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)
- ✓ Reaction tubes, at least 3 mL, Polypropylene/Polystyrol
- ✓ Water (deionized, distilled, or ultra-pure)
- ✓ Vortex mixer
- ✓ Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)

Precautions for Use

- ✓ 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. 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. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
5. 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. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
6. Duplicate determination of sample is highly recommended.
7. 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.
8. Incubation times do influence the results. All wells should be handled in the same order and time intervals.
9. To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
10. A calibrator curve must be established for each run.
11. The controls should be included in each run and fall within established confidence limits.
12. 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.

13. 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.
14. 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.
15. For information on hazardous substances included in the kit please refer to Safety Data Sheets (SDS). The Safety Data Sheets for this product is made available directly on the website of the manufacturer or upon request.
16. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
17. In case of any sever 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 with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

✓ Limitations

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

✓ Interfering substance

- Serum

Sample containing precipitates or fibrin stands 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.

- 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.

✓ Drug 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, ephidrine, guaifenesin, mephenesin (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, methyldopa, 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.

Assay Protocol

Reagent Preparation

- Wash Buffer
  Dilute the 20 mL Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 mL.
  Storage: 2 month 2–8°C.
- 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 and mix thoroughly.
- Serotonin Microtiter Strips
  In rare cases residues of 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.

Sample Preparation

✓ Sample collection and storage
  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, ephidrine, guaifenesin, mephenesin (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, methyldopa, 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.

- 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.

Samples containing anticoagulant 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; for longer periods (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.

✓ When stabilizing urine, consider the acidy (see *Interfering substance*).

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.

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.

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.

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.

Carry out the washing steps thoroughly! Poor washing might influence the results

- Sample preparation - Acylation

1. Pipette 20 µL of the standards, controls, and samples into the respective reaction tubes.

2. Add 500 µL of Acylation Buffer to all tubes.
3. Add 50 µL of Acylation Reagent 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).

Take 20 µL of the acylated standards, controls, and samples for the Serotonin ELISA.

- Serotonin ELISA

1. Pipette 20 µL of the acylated standards, controls and samples into the appropriate wells of the Serotonin Microtiter Strips.
2. Pipette 50 µL of the Serotonin Antiserum into all wells.
3. Incubate 60 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
4. Discard or aspirate the contents 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 all wells.
6. Incubate for 30 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).
7. Discard or aspirate the contents 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 all wells.
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 Solution to all wells and shake the microtiter plate shortly.
11. Read the absorbance of the solution in the wells within 10 minutes, using a microtiter plate reader set to 450 nm (If available a reference wavelength between 620 nm and 650 nm is recommended).

Data Analysis

Calculation of Results

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).

Note: 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 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:
µg/24h = µg/L x L/24h
The amount of Serotonin normalized to creatinine is calculated as following:
µg/g creatinine = ng/mL (serotonin) / creatinine (mg/dL) x 100

✓ Measuring range

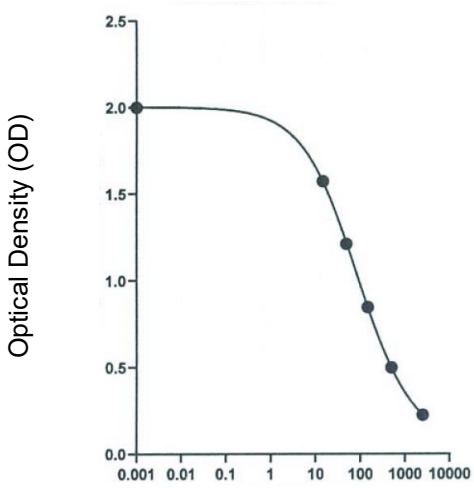
	Serotonin	
Measuring range	Serum	8 – 2170 ng/mL
	Urine	8 – 2027 ng/mL

✓ Conversion
Serotonin (ng/mL) x 5.67=Serotonin (nmol/L)

✓ Quality control

- Typical standard curve

  Example do not use for calculation.



Performance Characteristics

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.

✓ Sensitivity

- Limit of Blank (LOB): 2.9 ng/mL
- Limit of Detection (LOD): 5.9 ng/mL
- Limit of Quantitation (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-Hydroxytryptophane	<0.1

✓ Linearity

	Serial dilution up to	Mean (%)	Range (%)
Urine	1:64	98	88 - 111
Serum	1:64	103	93 -113

✓ Linear range

Serum	18 – 2170 ng/mL
Urine	20 – 2027 ng/mL

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

✓ Precision

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).

Intra-Assay (Serum)			Inter-Assay (Serum)		
Sample	Mean ± SD [ng/mL]	CV (%)	Sample	Mean ± SD [ng/mL]	CV (%)
1	11.8 ± 2.1	17.6	1	11.8 ± 3.3	28.2
2	61.6 ± 5.2	8.4	2	61.6 ± 7.7	12.5
3	102 ± 8.6	8.5	3	102 ± 12.3	12.1
4	227 ± 15.5	6.8	4	227 ± 23.0	10.1
5	493 ± 25.2	5.1	5	493 ± 55.7	11.3
6	1792 ± 109	6.1	6	1792 ± 165	9.2
Intra-Assay (Urine)			Inter-Assay (Urine)		
Sample	Mean ± SD [ng/mL]	CV (%)	Sample	Mean ± SD [ng/mL]	CV (%)
1	18.1 ± 2.0	11.3	1	18.1 ± 4.0	22.2
2	55.2 ± 4.0	7.3	2	55.2 ± 6.4	11.7
3	153 ± 9.1	5.9	3	153 ± 14.6	9.5
4	240 ± 11.4	4.8	4	240 ± 21.9	9.1
5	498 ± 29.3	5.9	5	498 ± 44.5	8.9
6	1798 ± 120	6.7	6	1798 ± 221	12.3

✓ Recovery

	Range [ng/mL]	Mean (%)	Range (%)
Urine	10.0-1023	91	82-98
Serum	49.4-1046	98	84-112

✓ Lot-to-Lot

	Sample	Mean ± SD [ng/mL]	CV (%)
Serotonin in urine (n = 3)	1	118 ± 8.5	7.2
	2	549 ± 47.3	8.6
Serotonin in serum (n = 3)	1	90.3 ± 2.0	2.2
	2	755 ± 52.2	6.9

Metrological Traceability – The values assigned to the standards and controls of the Serotonin ELISA are traceable to SI Unites by calibrated weighing with quality-controlled analyte.

Standards and Controls	Uncertainty [%]
	1.2%

✓ Serotonin ELISA

	Concentration [ng/mL]	Expanded Uncertainty [%] k = 2*
Serum	61.6	25.1
	227	20.3
	Concentration [ng/mL]	Expanded Uncertainty [%] k = 2*
Urine	18.1	44.5
	55.2	23.5
	153	19.2
	240	18.4
	498	18.0
	1798	24.7

* This defines an interval about the measured result that will include the true value with a probability of 95%

Resources

References

1. Bieger, W.P., NeuroStress Guide. 2011.
2. Chojnacki, C., et al., Evaluation of serotonin and dopamine secretion and metabolism in patients with irritable bowel syndrome. Pol Arch Intern Med, 2018. 128(11): p. 711 – 713.
3. Huang, H., Z. Chen, and X. Yan, Simultaneous determination of serotonin and creatinine in urine by combining two ultrasound-assisted emulsification microextractions with on-column stacking in capillary electrophoresis. J Sep Sci, 2012. 35(3): p. 436 – 44.
4. Piešťanský, J., K. Maráková, and P. Mikuš, Two-Dimensional Capillary Electrophoresis with On-Line Sample Preparation and Cyclodextrin Separation Environment for Direct Determination of Serotonin in Human Urine. Molecules, 2017. 22(10).
5. Lindström, M., et al., Comparison of serum serotonin and serum 5-HIAA LC-MS/MS assays in the diagnosis of serotonin producing neuroendocrine neoplasms: A pilot study. Clin Chim Acta, 2018. 482: p. 78 – 83.
6. Ren, C., et al., Low levels of serum serotonin and amino acids identified in migraine patients. Biochem Biophys Res Commun, 2018. 496(2): p. 267 – 273.
7. Moriarty, M., et al., Development of an LC-MS/MS method for the analysis of serotonin and related compounds in urine and the identification of a potential biomarker for attention deficit hyperactivity/hyperkinetic disorder. Anal Bioanal Chem, 2011. 401(8): p. 2481 – 93.
8. Nichkova, M.I., et al., Evaluation of a novel ELISA for serotonin: urinary serotonin as a potential biomarker for depression. Anal Bioanal Chem, 2012. 402(4): p. 1593 – 600.
9. Holck, A., et al., Plasma serotonin levels are associated with antidepressant response to SSRIs. J Affect Disord, 2019. 250: p. 65 – 70.

Plate Layout

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