Serotonin ELISA Kit

Catalog Number KA1894

96 assays

Version: 12

Intended for research use only

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Introduction

Intended Use

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

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.

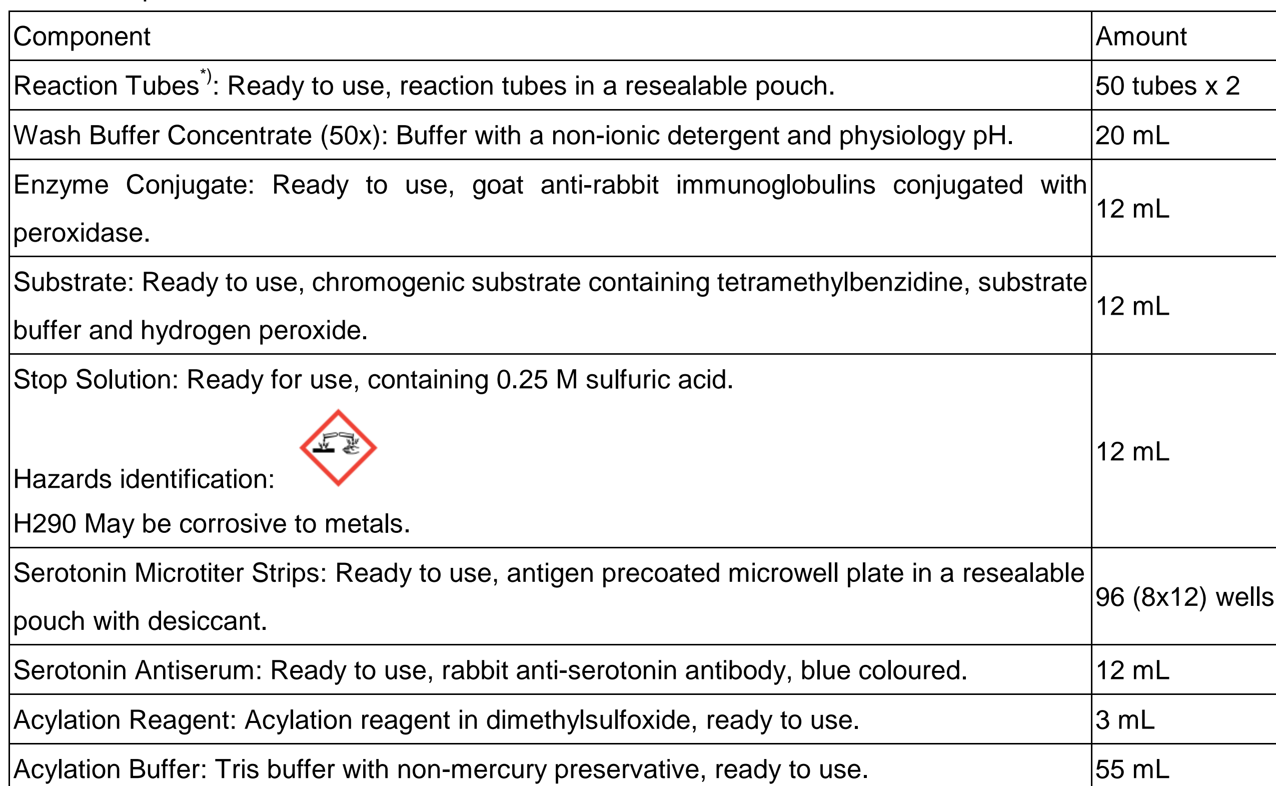
Principle of the Assay

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.

General Information

Materials Supplied

List of component



Component	Amount
Reaction Tubes*): Ready to use, reaction tubes in a resealable pouch.	50 tubes x 2
Wash Buffer Concentrate (50x): Buffer with a non-ionic detergent and physiology pH.	20 mL
Enzyme Conjugate: Ready to use, goat anti-rabbit immunoglobulins conjugated with peroxidase.	12 mL
Substrate: Ready to use, chromogenic substrate containing 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, antigen precoated microwell plate in a resealable pouch with desiccant.	96 (8x12) wells
Serotonin Antiserum: Ready to use, rabbit anti-serotonin antibody, blue coloured.	12 mL
Acylation Reagent: Acylation reagent in dimethylsulfoxide, ready to use.	3 mL
Acylation Buffer: Tris buffer with non-mercury preservative, ready to use.	55 mL

*) Instead of the Reaction tubes it is also possible to use 48 wells macrotiter plates for the sample preparation and acylation (please refer to “Sample preparation and acylation of serum, urine and platelets”).

Standards and Controls - Ready to use

Component	Colour/Cap	Concentration (ng/mL)	Concentration (nmol/mL)	Amount
Standard A	white	0	0	4 mL
Standard B	light yellow	15	85.1	4 mL
Standard C	orange	50	284	4 mL
Standard D	dark blue	150	851	4 mL
Standard E	light grey	500	2840	4 mL
Standard F	black	2500	14175	4 mL
Control 1	light green	Refer to vial labels for expected value and acceptable range.		4 mL
Control 2	dark 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 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.

Materials Required but Not Supplied

- ✓ 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

Note: 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.

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. 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 Material Safety Data Sheets (MSDS). The Material Safety Data Sheets 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.

✓ Limitations

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

✓ Interfering substance

- Serum/Plasma

  Sample containing precipitates or fibrin stands might cause inaccurate results.

  Hemolytic samples (up to 4 mg/mL hemoglobin), icteric sample (up to 50 mg/dL bilirubin) and lipemic sample (up to 1700 mg/dL triglycerides) have no influence on 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.

✓ Drug interferences: Please refer to “Sample Preparation”.

✓ 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: 1 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.
- 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
  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 and especially lipemic samples should not be used for the 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, 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 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.

Assay Procedure

Allow all reagent 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.

- 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).
   Note: Take 25 µL of the prepared standards, controls and samples for the Serotonin ELISA Kit.

- Serotonin ELISA
  The usage of a shaker is not mandatory. The alternative protocol without shaker is highlighted in italic and underline.

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.

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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).

Data Analysis

Calculation of Results

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).
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 for urine and serum samples can be read directly from the calibration curve.

✓ Measuring range: 10.2-2500 ng/mL

✓ 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/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)

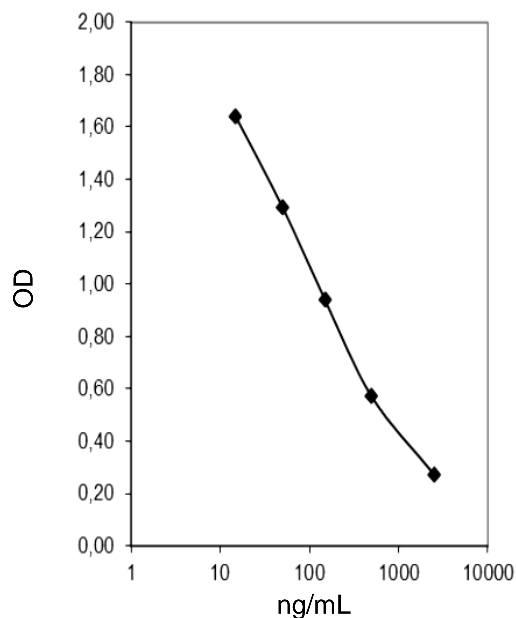
✓ Expected reference values
It is strongly recommended that each laboratory should determine its own reference values.

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

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

- Typical standard curve

  This example is a mean of 10 different runs; do not use for calculation.



Performance 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

✓ 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

✓ Precision

Intra-Assay				Inter-Assay			
	Sample	Range (ngmL) 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		-	-	-

✓ 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: $y = 0.85x + 33.18$; $R^2 = 0.97$

**Note: Commercial available RIA*

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Resources

References

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 inNonsegmental Vitiligo. Acta Dermatovenerol Croat, 20 (1): 14-20 (2012)
3. Ciprandi et al. Serotonin in Allergic Rhinitis: a Possible Role for Behavioural Symptomes, 10 (3): 183-188 (2011)

Plate Layout

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