5-HT / Serotonin Research ELISA Kit

Enzyme Immunoassay for the quantification of Serotonin in different sample volumes and biological sample types including serum, urine, platelets, platelet-poor plasma, tissue homogenates, dialysates and other samples.

Catalog number: ARG80482

Package: 96 wells

TABLE OF CONTENTS

SECTION **Page**

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PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification Serotonin in different biological samples and volumes.

This assay employs the competitive quantitative enzyme immunoassay technique. Serotonin is first quantitatively acylated.

The antigen has been pre-coated onto a microtiter plate. Acylated controls, standards or samples and the solid phase bound analytes compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. Anti-rabbit IgG conjugated to Peroxidase is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Serotonin present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Serotonin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Serotonin/5-HIAA coated microplate	12 strips X 8 wells	4°C
Standard A-F (0, 15, 50, 150, 500, 2500 ng/ml) (Standards have to be diluted 1+1000 with diluent, **working concentrations: 0, 0.015, 0.05, 0.15, 0.5, 2.5 ng/ml)**	4 ml each	4°C
Control 1 (100 ng/ml, control has to be diluted 1+1000 with diluent, **accept conc.: 0.1 ng/ml ± 40%)**	4 ml	4°C
Control 2 (300 ng/ml, control has to be diluted 1+1000 with diluent, **accept conc.: 0.3 ng/ml ± 40%)**	4 ml	4°C
Stabilizer	4 ml (Ready-to-use)	4°C
Acylation Buffer	4 ml (Ready-to-use)	4°C
Acylation Plate	1 X 96 wells (Ready-to-use)	4°C
20X Diluent	50 ml	4°C
Serotonin Antiserum	3 ml (Ready-to-use)	4°C
Anti-rabbit IgG-peroxidase conjugate	12 ml (Ready-to-use)	4°C
50X Wash Buffer	20 ml	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12 ml (Ready-to-use)	4°C
Adhesive foil	4 pieces	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620-650 nm as reference wavelength)
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the unopened reagents at 2 -8°C until expiration date. 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.
- 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.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- 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.
- Ensure complete reconstitution and dilution of reagents prior to use.
- The controls should be included in each run and fall within established

confidence limits. The confidence limits are listed in the QC-Report.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- In rare cases residues of the blocking and stabilizing reagent can be seen in the wells of Serotonin coated microtiter strips as small, white dots or lines. These residues do not influence the quality of the product.

SAMPLE COLLECTION & STORAGE INFORMATION

In general, this assay is dedicated for any biological samples such as serum, urine, platelets, platelet-poor plasma, tissue homogenates, dialysates and other samples.

Storage: Store at 2 – 8 °C for up to 6 hours. For longer period storage, aliquot and store samples at ≤ -20 °C for up to 6 months. Avoid repeated freeze-thaw cycles and exposure to direct sunlight.

To protect Serotonin against oxidative degradation, the samples should contain 1% Stabilizer reagent provided in this kit.

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.

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.

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.

Note: To protect Serotonin against oxidative degradation the samples should contain 1% Stabilizer (provided with this kit)

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20 ml of 50X Wash buffer into distilled water to a final volume of 1000 ml to yield 1X Wash buffer. Storage: up to one month at 2-8°C.
- 1X Diluent: Dilute 50 ml of 20X Diluent with distilled water to a final volume of 1000 ml. Storage: up to 1 months at 2-8°C. (The 1X Diluent buffer contains 1% Stabilizer, so does not need to add 1% Stabilizer in it.)
- Standards and controls: The standards and controls have to be diluted freshly 1+1000 with 1X diluent or buffer (If TRIS buffer is used, the buffer used for the respective experiment, enriched with 1% stabilizer).

  For example: 10 µl of standard + 10 ml of 1X Diluent.

  The standards and controls have to be prepared fresh prior to use.
- Samples: The Serotonin Research ELISA is a flexible high sensitive test system for various biological sample types and sizes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs.
  - Using poorly diluted or undiluted samples might lead to incorrect results due to a matrix effect. Therefore it is advisable to perform a linearity experiment prior to the test. Make different dilutions of a sample with the included diluent (e.g. 1:1, 1:4, 1:10, 1:20 and so on), spike each dilution with the same known concentration and check the recovery. If the samples are found consistently correct and no matrix effect is detected, samples can be used undiluted.
  - If a matrix effect is detected, samples should be diluted with the

included diluent prior to the test. It is also possible to dilute the standards with the sample matrix instead of diluent, in order to create the same matrix conditions for standards and samples. Currently the following buffers/solvents are evaluated for use: Ringer Buffer, PBS and 0.9% NaCl. If another substance is used, please check the compatibility by a Proof of Principle prior to the measurement. Prepare a stock solution of serotonin or use standard F. Add small amounts (to change the native sample matrix as little as possible) of the stock solution or standard F to the sample matrix and check the recovery. Please take the correction factor into account.

- If the expected sample concentrations are higher than the highest standard range, samples should be diluted accordingly with the diluent in this kit. Please take the correction factor into account.
- The measuring range and sensitivity of this test are defined by the correction factor, which is calculated by sample volume and dilution. If the expected concentrations are unknown, please test different dilutions and amounts of sample volume, to make sure that the samples will fall into the measuring range of this assay.
- Serotonin decomposes fast in acidic solution (< pH 3) and at higher temperatures.
- When acidic sample solutions are used, protect serotonin by keeping the temperature low (2-8 °C). Use pre-cooled buffers and materials. Adjust the pH to (6 -7.4) as soon as possible.
- A pH 7- 8.5 during acylation is mandatory.
- To protect serotonin against oxidative degradation add 1 % Stabilizer.

ASSAY PROCEDURE

Sample Preparation and Acylation

1. Pipette 100 µl of diluted standards, diluted controls and 1-100 µl samples into the appropriate wells of the Acylation Plate. (The wells of the Acylation Plate are covered by plastic bars which have to be removed prior to use.)
2. Add 1X diluent or buffer (TRIS buffer with 1% stabilizer, please refer to Standards and controls preparation section) to the wells containing samples to a final volume of 100 µl.
3. Add 25 µl of Acylation Buffer into all wells.
4. Incubate for 30 mins at RT on microplate shaker (~600 rpm)
5. Use 100 µl of the acylated standards, controls and samples from step 4 for Serotonin assay.

Serotonin ELISA procedure

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of the acylated standards, controls and samples into the appropriate wells of Serotonin/5-HIAA coated Microtiter Strips.
3. Add 25 µl of Serotonin Antiserum into wells.
4. Cover plate with Adhesive Foil, shake briefly and incubate for 15-20 hours at 2-8°C.
5. Remove the foil and discard. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After

the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

6. Add **100 µl** of **Anti-rabbit IgG-peroxidase conjugate** into wells.
7. Incubate for **30 mins at RT** on a microplate shaker (600 rpm).
8. Aspirate each well and **wash as step 5**.
9. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **20-30 mins at RT** with shaking (600rpm) on a microplate shaker. Avoid exposure to light.
10. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
11. **Read** the OD with a microplate reader **at 450 nm** (with a reference wavelength between 620nm and 650nm) within 10 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)

6. The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor:

- Controls: The concentrations can be read directly from the standard curve.
- Samples: The concentrations of samples have to be multiplied by a correction factor:

  Correction factor =

  100 µl (volume of standards) / sample volume (µl)

Example 1:

10 µl of the undiluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.

Correction factor = 100/10 = 10

Final concentration of the sample = 0.02 ng/ml x 10 = 0.2 ng/ml serotonin

Example 2:

100 µl of the 1:100 pre-diluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.

Correction factor = 100 X (100/100) =100

Final concentration of the sample = 0.02 ng/ml x 100 = 2.0 ng/ml serotonin

Example 3:

10 µl of the 1:100 pre-diluted sample are acylated and the concentration taken from the standard curve is 0.02 ng/ml serotonin.

Correction factor = 100 x (100/10) = 1000

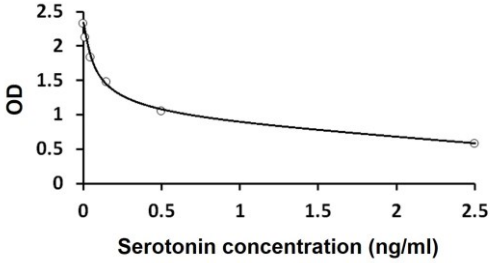
Final concentration of the sample = 0.02 ng/ml x 1000 = 20 ng/ml serotonin

7. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Serotonin (ng/ml)	0	0.015	0.05	0.15	0.5	2.5
Serotonin (nmol/L)	0	0.085	0.28	0.85	2.8	14
Conversion	Serotonin (ng/ml) x 5.67 = Serotonin (nmol/L)					

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

0.005 ng/ml * correction factor

Assay Range

0.015-2.5 ng/ml

Specificity

The tested cross-reactivity was listed as below:

Substance	Cross Reactivity (%)
Serotonin	100
Tryptamine	0.19
Melatonin	0.03
5-Hydroxyindole acetic acid	< 0.002
Phenylalanine	< 0.002
Histidine	< 0.002
Tyramine	< 0.002
5-Hydroxytryptophan	< 0.002