Human 5-HT / Serotonin ELISA Kit

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

Catalog number: ARG80480

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	Page
PRINCIPLE OF THE ASSAY	3
MATERIALS PROVIDED & STORAGE INFORMATION	4
MATERIALS REQUIRED BUT NOT PROVIDED	4
TECHNICAL HINTS AND PRECAUTIONS	5
SAMPLE COLLECTION & STORAGE INFORMATION	6
REAGENT PREPARATION	8
ASSAY PROCEDURE	9
CALCULATION OF RESULTS	10
EXAMPLE OF TYPICAL STANDARD CURVE	12
QUALITY ASSURANCE	12

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification Serotonin in Serum, urine, Plasma, PRP and platelets.

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-goat 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
Anti-rabbit IgG-peroxidase conjugate	12 ml (Ready-to-use)	4°C
Standard A-F (0, 15, 50, 150, 500, 2500 ng/ml)	4 ml each (Ready-to-use)	4°C
Control 1 (100 ng/ml ±40%)	4 ml (Ready-to-use)	4°C
Control 2 (300 ng/ml ±40%)	4 ml (Ready-to-use)	4°C
Serotonin Antiserum	6 ml (Ready-to-use)	4°C
Acylation Buffer	55 ml (Ready-to-use)	4°C
Acylation Reagent	2 X 3 ml (Ready-to-use)	RT
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

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: reference wavelength at 620nm - 650nm)
- Pipettes and pipette tips
- Deionized or distilled water
- Orbital microplate shaker: 3 mm (0.1118 in) 600 ± 10 rpm or 19 mm (0.75 in) 170 ± 10 rpm.
- Reaction tubes, at least 3 ml, Polypropylene/Polystyrol.
- 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 kit at 2-8°C at all times. Opened reagents are stable for up to 2 months at 2-8°C
- 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.
- 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.
- If crystals are observed in the 50X Wash buffer, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- 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.
- In rare cases residues of the blocking and stabilizing reagent can be seen in the wells of Serotonin Microtiter Strips as small, white dots or lines. These residues do not influence the quality of the product.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Note: Foods or medications might influence the Serotonin level in samples. So Serotonin containing foods such as pineapple, eggplant, avocados, bananas, currants, kiwis, melon, mirabelles, plums, peaches chocolate, gooseberries, tomatoes, walnuts and alcohol, caffeine, nicotine should be avoided 2-4 days before and during the day of the sample collection (24-hour urine).

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. Repeated freezing and thawing of samples should be avoided.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C (Storage up to 3 days at 2-8°C; it is recommended store at ≤-20°C for longer storage period (up to 6 months). Avoid repeated freeze-thaw cycles. Haemolytic and especially lipemic

samples should not be used with this assay.

Urine: Spontaneous or 24-hour urine, collected in a bottle containing 10-15ml of 6M HCl can be used (10 µl 100% acetic acid per 1 ml of urine sample). Storage: Storage up to 3 days at 2-8°C, or store at -20°C for longer period (up to 6 months). Avoid exposure to sunlight.

Platelets: More than 98% of the circulating serotonin is located in the platelets and is released during blood clotting. Blood must be collected by venipuncture in plastic tubes containing EDTA or Citrate.

- Platelet-rich Plasma (PRP): To obtain PRP, the samples are centrifuged for 10 minutes at RT (200 x g). Transfer supernatant to another tube and count the platelets.
- Platelets: The platelet pellet is obtained by adding 800 µl of physiological saline to 200 µl PRP (Platelet-Rich Plasma, containing between 350,000 – 500,000 platelets/µl) and centrifuge at 4,500 x g, 10 minutes at 4°C. The supernatant is then discarded. 200µl of distilled water is added to the pellet and mixed thoroughly on a vortex mixer. The suspension can be stored at -20°C for several weeks. After thawing the frozen samples, centrifuge at 10,000 x g for 2 minutes at RT. **20 µl** of supernatant is used for the acylation reaction.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 20 ml of **50X** Wash buffer into 980 ml of **distilled water** to yield 1X Wash buffer with a final volume of 1000 ml. Storage: up to 2 months at 2-8°C.
- **Acylation Reagent:** The acylation reagent has a freezing point of 18.5°C. To ensure that the acylation reagent is in liquid form when being used, it must be ensured that the acylation reagent has reached RT and forms a homogenous, crystal-free solution. If more than 3 ml are needed, pool the contents of the individual vials of Acylation Reagent and mix thoroughly before use.

ASSAY PROCEDURE

Sample Preparation and Acylation

1. Pipette **20 µl** of **standards, controls and samples** into the respective Reaction tubes.
2. Add **500 µl** of **Acylation Buffer** into all tubes.
3. Add **50 µl** of **Acylation Reagent** into all tubes.
4. Brief vortex to **mix** thoroughly and incubate for **15 mins at RT (20-25°C)**.
5. Add **500 µl** of **distilled water** into all tubes.
6. Use **20 µl** of the **acylated standards, controls and samples** for Serotonin ELISA procedure.

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 **20 µl** of the **acylated standards, controls and samples** into the appropriate wells of **Serotonin/5-HIAA coated Microtiter Strips**.
3. Add **50 µl** of **Serotonin Antiserum** into wells.
4. Incubate for **60 mins** at **RT** on a microplate shaker (600rpm).
5. Aspirate each well and wash, repeating the process 3 times for a **total 4 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 (600rpm).
8. Aspirate each well and **wash as step 5**.
9. Add **100 µl** of **TMB substrate** solution into each well. Incubate for **20-30 min at RT** with shaking (600rpm). 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** (optional: read with a reference wavelength between 620nm and 650nm) within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

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

2. Using semi-logarithmic 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 details. (https://www.arigobio.com/elisa-analysis)

6. If the initial assay found samples contain Serotonin higher than the highest standard (Standard F, 2500 ng/ml), the samples can be diluted with Standard A (0 ng/ml) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

7. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Serotonin (ng/ml)	0	15	50	150	500	2500
Serotonin (nmol/L)	0	85.1	284	851	2840	14175
Conversion	Serotonin (ng/ml) x 5.67 = Serotonin (nmol/L)					

- Controls, urine samples, serum samples: The concentrations can be read directly from the standard curve.
- Urine samples:
  - 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
- Platelets Samples: The content of serotonin in platelets is referred to 10^9 platelets.

  Example:

  Measured Serotonin concentration: 200 ng/ml

  Number of the platelets in the PRP: 300,000 / µl

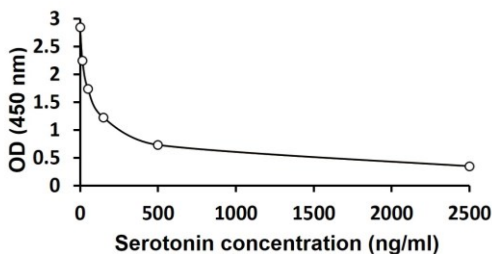
  = 0.3 x 10^9 platelets/ml with serotonin content of 200 ng.

The resulting serotonin content in the platelets is:

666 ng/ 10^9 platelets (200 ng serotonin x 1.0 x 10^9 /0.3 x 10^9)

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

Limit of Blank (LOB): 2.9 ng/ml

Limit of Detection (LOD): 5.9 ng/ml

Limit of Quantitation (LOQ): 8.0 ng/ml

Assay Range

Standard range: 15-2500 ng/ml

Measuring range:

Serum: 8 - 2170 ng/ml

Urine: 8- 2027 ng/ml

Specificity

No significant cross-reactivity was found for the following factors:

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

Intra-assay and Inter-assay precision

The CV value of intra-assay precision were: Serum: 8.75%, Urine: 8.38% and CV value of inter-assay precision were: Serum: 13.9%, Urine: 12.3%.

Recovery

82-98% (Urine)

84-112% (Serum)

Linearity

88-111% (Urine)

93-113% (Serum)

Linear range

Serum: 18 - 2170 ng/ml

Urine: 20- 2027 ng/ml