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## Instructions for use

# 5-HIAA ELISA

REF **IB89129R**

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

**5-HIAA ELISA**

## 1. Introduction

### 1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of 5-Hydroxy-3-Indole Acetic Acid (5-HIAA) in urine.

First, 5-HIAA is derivatized by methylation. The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The methylated analyte in the standards, controls and samples and the solid phase bound analyte compete for a fixed number of antibody binding sites. After the system has reached equilibrium, free antigen and free antigen-antibody 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 standard curve prepared with known standard concentrations.

### 1.2 Background

5-HIAA (5-hydroxyindoleacetic acid) is the major urinary metabolite of serotonin, an ubiquitous bioactive amine. Serotonin, and consequently 5-HIAA, is produced in excess by most carcinoid tumors, especially those associated with the carcinoid syndrome. The syndrome includes flushing and diarrhea, and, less frequently, heart failure and bronchoconstriction. Quantitation of urinary 5-HIAA is therefore intended to test for carcinoid.

## 2. Procedural cautions, guidelines, warnings and limitations

### 2.1 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 standard 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 Safety Data Sheet (SDS). The Safety Data Sheet 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 according to national regulations.

### 2.2 Limitations

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

### 2.2.1 Interfering substances

**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.

### 2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of 5-HIAA level in the sample.

### 2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

## 3. Storage and stability

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. Make sure that the Methylation Reagent is recapped immediately after pipetting.

## 4. Materials

### 4.1 Content of the kit

**BA D-0090** FOILS **Adhesive Foil** - Ready to use
Contents: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

**BA D-0023** REAC-TUBES **Reaction Tubes** - Ready to use
Contents: 2 x 50 tubes in a resealable pouch

**BA D-0024** REAC-PLATE **Reaction Plate** - Ready to use
Contents: 1 x 96 well plate, empty in a resealable pouch

**BA E-0030** WASH-CONC 50x **Wash Buffer Concentrate -** Concentrated 50x
Contents: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 ml/vial, light purple cap

**BA E-0040** CONJUGATE **Enzyme Conjugate** - Ready to use
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 ml/vial, red cap

**BA E-0055** SUBSTRATE **Substrate** - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 ml/black vial, black cap

**BA E-0080** STOP-SOLN **Stop Solution** - Ready to use
Contents: 0.25 M sulfuric acid
Volume: 1 x 12 ml/vial, light grey cap
Hazards identification:

H290 May be corrosive to metals.

**BA E-0931** MTP SER 5-HIAA **5-HIAA Microtiter Strips - Ready to use**
Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant

**BA E-1910** 5-HIAA-AS **5-HIAA Antiserum** - Ready to use
Contents: Rabbit anti – 5-HIAA antibody, blue coloured
Volume: 1 x 6 ml/vial, blue cap

**Standards** and **Controls** - Ready to use

| Cat. no. | Component | Colour/Cap | Concentration mg/l | Concentration µmol/l | Volume/ Vial |
|---|---|---|---|---|---|
| **BA E-1901** | STANDARD A | white | 0 | 0 | 4 ml |
| **BA E-1902** | STANDARD B | light yellow | 0.5 | 2.63 | 4 ml |
| **BA E-1903** | STANDARD C | orange | 1.5 | 7.88 | 4 ml |
| **BA E-1904** | STANDARD D | dark blue | 5 | 26.3 | 4 ml |
| **BA E-1905** | STANDARD E | light grey | 15 | 78.8 | 4 ml |
| **BA E-1906** | STANDARD F | black | 50 | 262.5 | 4 ml |
| **BA E-1951** | CONTROL 1 | light green | Refer to QC-Report for expected value and acceptable range! | | 4 ml |
| **BA E-1952** | CONTROL 2 | dark red | | | 4 ml |

Conversion: 5-HIAA (mg/l) x 5.25 = 5-HIAA (µmol/l)

Contents: Acidic buffer spiked with defined quantity of 5-HIAA

**BA E-0041** DILUENT **Diluent –** Ready to use

Contents: Acidic buffer with non-mercury preservatives

Volume: 1 x 22 ml/vial, white cap

**BA E-1913** ASSAY-BUFF **Assay Buffer –** Ready to use

Contents: TRIS containing buffer with non-mercury preservative

Volume: 2 x 55 ml/vial, dark green cap

**BA E-1937** METHYL-BUFF **Methylation Buffer** - Ready to use

Contents: Methanol and dimethylformamide

Volume: 1 x 11 ml/vial, brown cap

Hazards identification:

H226 Flammable liquid and vapour.
H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled
H360D May damage fertility or the unborn child.
H370 Causes damage to organs (eyes).
H319 Causes serious eye irritation.
H312 + H332 Harmful in contact with skin or if inhaled.

**BA E-1939** METHYL-REAG **Methylation Reagent** – Ready to use

Contents: Methylation reagent in diethyl ether

Volume: 1 x 2.25 ml, white cap

Hazards identification:

H225 Highly flammable liquid and vapour.
H302 Harmful if swallowed.
H370 Causes damage to organs.
H330 Fatal if inhaled.
H336 May cause drowsiness or dizziness.
H350 May cause cancer.



### 4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 20 - 300 µl; 1 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Ventilated hood
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

## 5. Sample collection and storage

Spontaneous urine or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, can be used.
If 24-hour urine is used please record the total volume of the collected urine.
Storage: for longer periods (up to 6 month) at -20 °C.
Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

## 6. Test procedure

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use.
Duplicate determinations are 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 absorption values may vary if a thermostat is not used. 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 Methylation Reagent is highly volatile. If possible, please pipette the Methylation Reagent with a repetitive pipette and make sure that the vial is recapped immediately after pipetting.*

### 6.1 Preparation of reagents

**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 at 2 - 8 °C

**5-HIAA 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.

### 6.2 Predilution of the standards, controls and samples

| | |
|---|---|
| **1.** | Pipette **50 µl** of **standards, controls** and **urine samples** into the respective wells of the **Reaction Plate.** |
| **2.** | Pipette **200 µl** of the **Diluent** into all wells. |
| **3.** | Shake for **1 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).<br>**20 µl** are needed for the **methylation.** |

### 6.3 Methylation

| | |
|---|---|
| **1.** | Pipette **20 µl** of the **prediluted standards**, **controls** and **urine samples** into the respective **Reaction Tubes.** |
| ⚠ | *The following steps 2-5 have to be performed in a ventilated hood!* |
| **2.** | Pipette **100 µl** of **Methylation Buffer** into all tubes. |
| **3.** | Add **20 µl** of **Methylation Reagent** to each tube and **mix each tube immediately after addition of the Methylation Reagent.** |
| **4.** | Cover all tubes and **methylate** for **20 min** at **RT** (approx. 20 °C). |
| **5.** | Pipette **1000 µl** of **Assay Buffer** into all tubes.<br>*After this step the use of a ventilated hood is not necessary any more!* |
| ⚠ | **Proceed** with the **ELISA** (Chapter 6.4) **immediately** as the methylated standards, controls and samples are only stable for 1 h! |

### 6.4 5-HIAA ELISA

| | |
|---|---|
| **1.** | Pipette **25 µl** of the **methylated standards, controls** and **samples** into the appropriate wells of the **5-HIAA Microtiter Strips.** |
| **2.** | Pipette **50 µl** of the **5-HIAA Antiserum** into all wells. |
| **3.** | Cover plate with **Adhesive Foil** and incubate for **1 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). |
| **4.** | Remove the foil. Discard or aspirate the content of the wells. Wash the plate **4 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 **Enzyme Conjugate** into all wells. |
| **6.** | Cover plate with **Adhesive Foil** and incubate for **1 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). |
| **7.** | Remove the foil. Discard or aspirate the content of the wells. Wash the plate **4 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 and incubate for **20 - 30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sunlight!*** |
| **9.** | Add **100 µl** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution. |
| **10.** | **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). |

## 7. Calculation of results

| **Measuring range** | **5-HIAA** |
|---|---|
| | 0.17 – 50 mg/l |

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).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *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.*

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

The total amount of 5-HIAA excreted in urine during 24 h is calculated as following:
**mg/24h = mg/l x l/24h**

**Conversion**
5-HIAA (mg/l) x 5.25 = 5-HIAA (µmol/l)

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

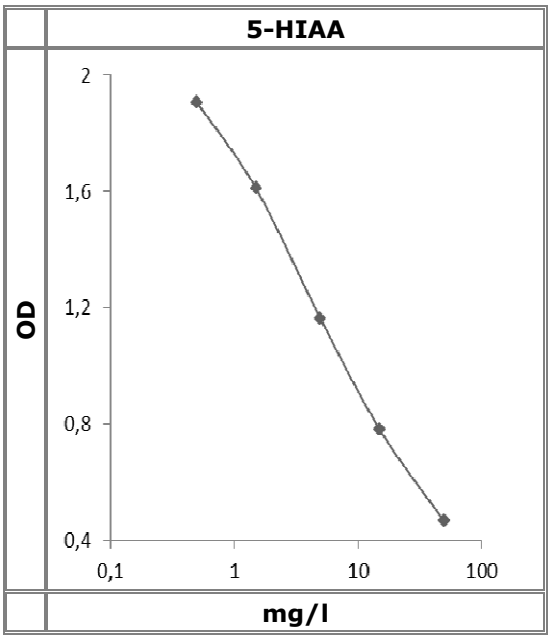
| | **5-HIAA** |
|---|---|
| 24-hour urine | < 15 mg/day |

### 7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

### 7.2 Typical standard curve

⚠ *Example, do not use for calculation!*




## 8. Assay characteristics

| **Analytical Sensitivity (Limit of Detection)** | **5-HIAA** |
|---|---|
| | 0.17 mg/l |

| **Analytical Specificity (Cross Reactivity)** | **Substance** | **Cross Reactivity (%)** |
|---|---|---|
| | | **5-HIAA** |
| | 5-HIAA | 100 |
| | Serotonin | 5.5 |
| | 5-Hydroxy-DL-Tryptophan | 1.8 |
| | Tryptamine | < 0.1 |
| | Melatonin | < 0.1 |
| | 5-Hydroxytryptamin | < 0.1 |
| | Vanillic mandelic acid | < 0.1 |
| | Homovanillic Acid | < 0.1 |

| **Precision** | | | | | |
|---|---|---|---|---|---|
| **Intra-Assay** | | | **Inter-Assay** | | |
| Sample | Range (mg/l) | CV (%) | Sample | Range (mg/l) | CV (%) |
| 1 n = 40 | 1.7 ± 0.2 | 14.1 | 1 n = 9 | 3.1 ± 0.3 | 8.6 |
| 2 n = 38 | 6.6 ± 0.6 | 8.6 | 2 n = 9 | 7.3 ± 0.8 | 10.8 |
| 3 n = 40 | 18.4 ± 1.9 | 10.3 | 3 n = 9 | 19 ± 2.2 | 11.4 |

| **Linearity** | | Range | Serial dilution up to | Range (%) |
|---|---|---|---|---|
| | 5-HIAA | 2.4 – 24.3 mg/l | 1:10 | 98 - 112 |

| **Recovery** | | Mean (%) | Range (%) | % Recovery after spiking |
|---|---|---|---|---|
| | 5-HIAA | 101 | 93 - 111 | |

| **Method Comparison versus HPLC** | 5-HIAA | HPLC = 0.9 ELISA + 0.2 | r = 0.99; n = 47 |
|---|---|---|---|

## 9. References/Literature


(1) Beer et al. Acupuncture for Hot Flashes in Patients With Prostate Cancer Patients. Urology, 76(5):1182–1188 (2010)

(2) Korse et al. Chromogranin A as an Alternative to 5-Hydroxyindoleacetic Acid in the Evaluation of Symptoms during Treatment of Patients with Neuroendocrine Tumors. Neuroendocrinology, 89:296–301 (2008)

(3) van Tuyl et al. Detection of small-bowel neuroendocrine tumors by video capsule endoscopy. Gastrointestinal Endoscopy, 64 (1):66-72 (2006)




⚠ ***For updated literature or any other information please contact your local supplier.***

**Symbols:**

| | | | | | |
|---|---|---|---|---|---|
|  | Storage temperature |  | Manufacturer |  | Contains sufficient for <n> tests |
|  | Expiry date | LOT | Batch code | | |
|  | Consult instructions for use | CONT | Content | | |
|  | Caution | REF | Catalogue number | RUO | For research use only! |