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LDN®

## Instructions for use

# 5-HIAA ELISA

REF BA E-1900

96 +2 +8 °C IVD CE

## 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 quantitatively 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 derivatized 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.

## 2. Advice on handling the test

### 2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

### 2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

### 2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

### 2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

### 2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

### 2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

## 3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

### 4.1. Contents of the kit

| | | | | |
|---|---|---|---|---|
| **BA D-0023** | REAC-TUBES | **Reaction Tubes** | 2 x 50 | ready for use |
| **BA D-0024** | REAC-PLATE | **Reaction Plate** | 1 x 96 wells | ready for use |
| **BA D-0090** | FOILS | **Adhesive Foil** | 1 x 4 | ready for use |
| **BA E-0030** | WASH-CONC 50x | **Wash Buffer Concentrate** | 1 x 20 mL | Concentrate. Dilute content with dist. water to a final volume of 1000 mL |
| **BA E-0040** | CONJUGATE | **Enzyme Conjugate** | 1 x 12 mL | ready for use, anti-rabbit IgG conjugated with |
| **BA E-0041** | DILUENT | **Diluent** | 1 x 22 mL | ready for use |
| **BA E-0055** | SUBSTRATE | **Substrate** | 1 x 12 mL | ready for use, containing a solution of |
| **BA E-0080** | STOP-SOLN | **Stop Solution** | 1 x 12 mL | ready for use, containing 0.25 M sulphuric acid |
| **BA E-1901** | STANDARD A | **Standard A** | 1 x 4 ml | ready for use |
| **BA E-1902** | STANDARD B | **Standard B** | 1 x 4 ml | ready for use |
| **BA E-1903** | STANDARD C | **Standard C** | 1 x 4 ml | ready for use |
| **BA E-1904** | STANDARD D | **Standard D** | 1 x 4 ml | ready for use |
| **BA E-1905** | STANDARD E | **Standard E** | 1 x 4 mL | ready for use |
| **BA E-1906** | STANDARD F | **Standard F** | 1 x 4 mL | ready for use |
| **BA E-1910** | 5-HIAA-AS | **5-HIAA Antiserum** | 1 x 6 mL | from rabbit, ready for use, blue coloured, blue screw cap |
| **BA E-1913** | ASSAY-BUFF | **Assay Buffer** | 2 x 55 mL | ready for use |
| **BA E-0931** | TUT SER 5-HIAA | **Serotonin-5-HIAA Microtiter Strips** | 1 x 96 wells | 12 strips, 8 wells each, break apart, pre-coated |
| **BA E-1937** | METHYL-BUFF | **Methylation Buffer** | 1 x 11 mL | ready for use |
| **BA E-1939** | METHYL-REAG | **Methylation Reagent** | 1 x 2.25 mL | ready for use |
| **BA E-1951** | CONTROL 1 | **Control 1** | 1 x 4 mL | ready for use |
| **BA E-1952** | CONTROL 2 | **Control 2** | 1 x 4 mL | ready for use |

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

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

## 5. Sample collection and storage

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, may be used.
Storage: for longer periods (up to 6 months) at -20°C.
Repeated freezing and thawing should be avoided.
Avoid exposure to direct sunlight.

## 6. Test procedure

Allow reagents and samples to reach room temperature.
The measurement in duplicates is recommended.

### 6.1 Preparation of reagents

**Wash Buffer**
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 2–8°C

### 6.2 Predilution of the 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** (18-25°C) on a shaker (approx. 600 rpm). **20 µL** are needed for the **methylation** |

### 6.3 Methylation

| | |
|---|---|
| **1.** ⚠ | Pipette **20 µL** of the **prediluted standards A - F**, **Control 1 & 2** and **urine** into the respective **Reaction Tubes.**<br>*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 minutes** at room temperature (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 hour! |

### 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 hour** at **RT** (20-25°C) on a shaker (approx. 600 rpm). |
| **4.** | Remove the foil. Discard or aspirate the content of the wells and **wash** each well **4 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 hour** at **RT** (20-25°C) on a shaker (approx. 600 rpm). |
| **7.** | Remove the foil. Discard or aspirate the content of the wells and **wash** each well **4 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 sun light!*** |
| **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** with a reference wavelength between 620 nm and 650 nm. |

## 7. Calculation of results

| | Concentration of the standards | | | | | |
|---|---|---|---|---|---|---|
| **Standard** | **A** | **B** | **C** | **D** | **E** | **F** |
| **5-HIAA (mg/L)** | 0 | 0.5 | 1.5 | 5 | 15 | 50 |
| **5-HIAA (µmol/L)** | 0 | 2.625 | 7.875 | 26.25 | 78.75 | 262.5 |
| **Conversion:** | 5-HIAA (mg/L) x 5.25 = 5-HIAA (µmol/L) | | | | | |

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).
The concentrations of the samples are read directly from the standard curve.

### 7.1 Quality control

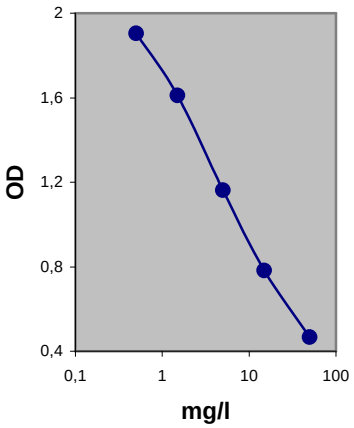
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

### 7.2 Calibration

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. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

### 7.3 Typical calibration curve




⚠ *Example. Do not use for calculation!*

## 8. Assay characteristics

| Expected Reference Values | | 5-HIAA |
|---|---|---|
| | Urine | < 15 mg/day |

| 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 | 100 | 93-110 | |

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

⚠ ***For current literature, information about clinical significance or any other information please contact your local supplier.***

**Symbols:**

| | | | | | |
|---|---|---|---|---|---|
|  | Storage temperature |  | Manufacturer |  | Contains sufficient for <n> tests |
|  | Expiry date | LOT | Batch code | I V D | For in-vitro diagnostic use only! |
|  | Consult instructions for use | CONT | Content | CE | CE labelled |
|  | Caution | REF | Catalogue number | RUO | For research use only! |