Human 5-HIAA ELISA Kit

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

Catalog number: ARG80441

Package: 96wells

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MANUFACTURED BY:

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PRINCIPLE OF THE ASSAY

This is an enzyme Immunoassay for the quantification of 5-Hydroxy-3-Indole Acetic Acid (5-HIAA) in urine samples.

This assay employs the competitive quantitative enzyme immunoassay technique. First, 5-HIAA is quantitatively derivatized by methylation. The antigen has been pre-coated onto a microtiter plate. Derivatized 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 5-HIAA 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 5-HIAA 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
Adhesive foil	1 X 4 pieces	RT

50X Wash Buffer	1 X 20ml	4°C
Anti-rabbit IgG-peroxidase conjugate	1 X 12ml (Ready-to-use)	4°C
TMB substrate	1 X 12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	1 X 12ml (Ready-to-use)	4°C
Diluent	22ml (Ready-to-use)	4°C
Standard A-F	4ml each (Ready-to-use)	4°C
5-HIAA Antiserum	6ml (Ready-to-use)	4°C
Reaction Plate	1 X 96 wells	4°C
Assay Buffer	2 X 55ml (Ready-to-use)	4°C
Control 1	4ml (Ready-to-use)	4°C
Control 2	4ml (Ready-to-use)	4°C
Methylation Buffer	11ml (Ready-to-use)	4°C
Methylation Reagent	2.25ml (Ready-to-use)	4°C
Reaction Tubes	2 X 50 tubes	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 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 kit at 4°C at all times.

- Briefly spin down all vials before use.
- If crystals are observed in the 50X Wash buffer, warm to RT (not more than 50°C) 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.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Urine – Spontaneous or 24-hour urine, collected in a bottle containing 10-15ml of 6M HCl. For linger storage, aliquot and store samples at ≤ -20 °C (up to 6 months). Avoid repeated freeze-thaw cycles. Avoid exposure to direct sunlight.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. Storage: up to 6 months at 4-8°C.

ASSAY PROCEDURE

Predilution of samples

1. Pipette 50 µl of standards, controls, and urine samples into appropriate wells of the reaction plate.
2. Pipette 200 µl of the diluent into all wells.
3. Shake for 1 min at RT on a shaker (600rpm). 20 µl are needed for methylation.

Methylation

1. Pipette 20 µl of prediluted standards, controls and urine samples into the respective reaction tubes. (Step 2-5 has to be performed in a ventilated hood).
2. Add 100 µl Methylation buffer to all tubes.
3. Add 20 µl Methylation Reagent into each tube.
4. Mix reaction tubes thoroughly immediately.
5. Cover tubes and methylate for 20 minutes at RT. Pipette 1000 µl of Assay buffer into all tubes.
6. **Proceed to ELISA assay immediately as the methylated standards, controls and samples are stable only for 1 hour.**

5-HIAA 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 25 µl methylated standards, controls, and samples into appropriate

wells of the 5-HIAA Microtiter Strips.

3. Add 50 µl of 5-HIAA Antiserum into all wells.
4. Cover plate with foil, incubate for 1 hour at RT on a shaker (600rpm).
5. Remove the foil and discard. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350 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 1 hour at RT on a 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 mins 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 450nm (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 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.

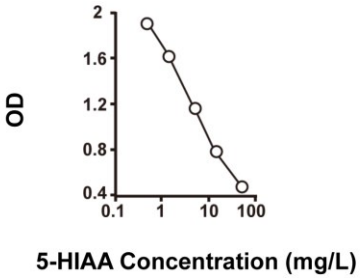
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. Refer to the table below for molar conversion:

	Concentration of 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	2.65	7.87	26.25	78.75	262.5
Conversion	5-HIAA (mg/L) x 5.25 = 5-HIAA (µmol/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

0.17 mg/L

Assay Range

0.5-50 mg/L

Specificity

No significant cross-reactivity was found for the following factors: Serotonin, 5-Hydroxy-DL-Tryptophan, Tryptamine, Melatonin, 5-Hydroxytryptamine, Vanillic mandelic acid, Homovanillic acid.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 8.6-14.1% and CV value of inter-assay precision was 8.6-11.4%.

Recovery

93-110%

Linearity

98-112%