5-HIAA (urine) ELISA Kit

Cat. No.:DEIA1973
Pkg.Size:96T

Intended use

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

General Description

5 Hydroxyindole acetic acid (5-HIAA) is a metabolite of serotonin that is excreted in the urine. Elevated levels of 5 Hydroxyindole acetic acid may indicate carcinoid tumour, while low levels may be associated with Cockayne Syndrome.

Principle Of The Test

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.

Reagents And Materials Provided

1. **Reaction Tubes:** 2 x 50, ready for use
2. **Reaction Plate:** 1 x 96 wells, ready for use
3. **Adhesive Foil:** 1 x 4, ready for use
4. **Wash Buffer Concentrate:** 1 x 20 mL, Concentrate. Dilute content with dist. water to a final volume of 1000 mL
5. **Enzyme Conjugate:** 1 x 12 mL, ready for use, anti-rabbit IgG conjugated with peroxidase
6. **Diluent:** 1 x 22 mL, ready for use
7. **Substrate:** 1 x 12 mL, ready for use, containing a solution of tetramethylbenzidine (TMB)
8. **Stop Solution:** 1 x 12 mL, ready for use, containing 0.25 M sulphuric acid
9. **Standard A:** 1 x 4 ml, ready for use
10. **Standard B:** 1 x 4 ml, ready for use
11. **Standard C:** 1 x 4 ml, ready for use
12. **Standard D:** 1 x 4 ml, ready for use
13. **Standard E:** 1 x 4 mL, ready for use
14. **Standard F:** 1 x 4 mL, ready for use
15. **5-HIAA Antiserum:** 1 x 6 mL, from rabbit, ready for use, blue coloured, blue screw cap
16. **Assay Buffer:** 2 x 55 mL, ready for use
17. **Serotonin-5-HIAA Microtiter Strips:** 1 x 96 wells 12 strips, 8 wells each, break apart, pre-coated
18. **Methylation Buffer:** 1 x 12 mL, ready for use
19. **Methylation Reagent:** 1 x 2.25 mL, ready for use
20. **Control 1:** 1 x 4 mL, ready for use
21. **Control 2:** 1 x 4 mL, ready for use

Materials Required But Not Supplied

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

Storage

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.

Specimen Collection And Handling

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.

Reagent Preparation

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

Assay Steps

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

Methylation
1. Pipette 20 µL of the prediluted standards A - F, Control 1 & 2 and urine 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 minutesat room temperature (approx. 20 C).
5. Pipette 1000 µL of Assay Buffer into all tubes.
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!

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).

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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.

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.

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

Calculation

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.

	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)					

Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each assay plate.

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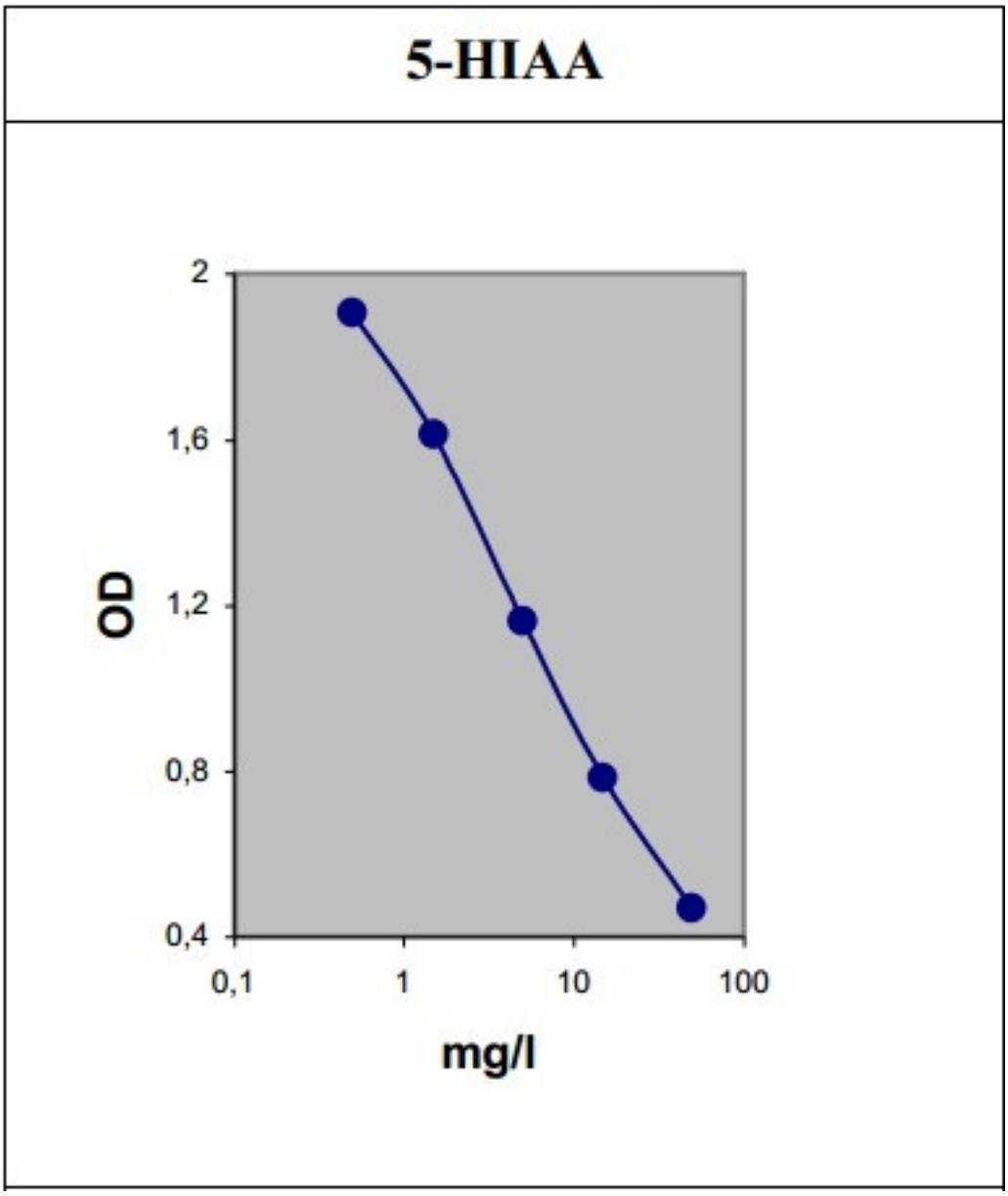

Reference Values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

Expected Reference Values		5-HIAA
	Urine	3 - 15 mg/day

Sensitivity

The minimum detectable dose of 5-HIAA is 0.17 mg/L.

Specificity

5-HIAA: 100 %

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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 %

Linearity

5-HIAA: 98 - 112 %

Recovery

5-HIAA Urine: 93-110 %

Reproducibility

The within assay variability and between assay variability are shown below:

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

Interferences

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.

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.

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