
Instructions for use
5-HIAA ELISA



KAPL10-1900



5-HIAA ELISA

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-1937	METHYL-BUFF	Methylation Buffer	1 x 12 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 .  <i>The following steps 2-5 have to be performed in a ventilated hood!</i>
2.	Pipette 100 µL of Methylation Buffer into all tubes.
3.	Add 20 µL of Methylation Reagent to each tube and <u>mix each tube immediately after addition of the Methylation Reagent.</u>
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. <i>After this step the use of a ventilated hood is not necessary any more!</i>
	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). <i>Avoid exposure to direct sun light!</i> 
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:	$5\text{-HIAA (mg/L)} \times 5.25 = 5\text{-HIAA (µmol/L)}$					

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.

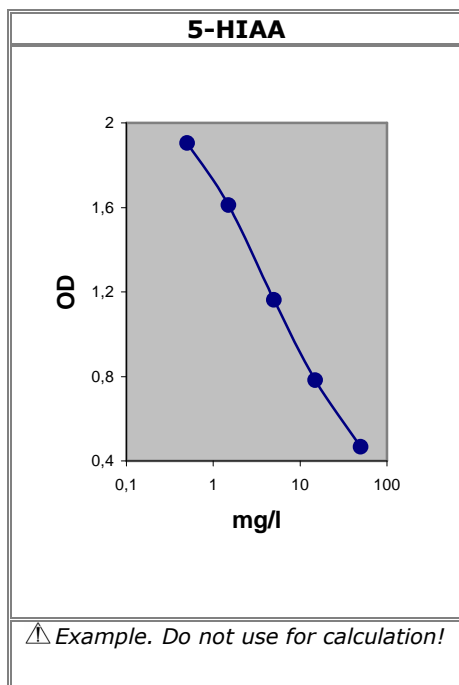
7.2 Calibration

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















8. Assay characteristics

Expected Values	Reference	5-HIAA			
	Urine	3 - 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 actual 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		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!