

# 5 – HIAA

Chromatographic – Colorimetric Determination of 5-Hydroxy-3-Indoleacetic Acid (5-HIAA) and Serotonin in Urine

20 tests

REF KR07-20

## INTENDED USE

Kit for quantitative *in vitro* determination of 5-Hydroxy-3-Indoleacetic Acid and Serotonin in urine.

## PRINCIPLE

The final products of tryptophane metabolism are adsorbed on a neutral resin. After the washing of interfering substances, serotonin (5HT), 5-hydroxy-tryptophane (5HTP) and 5-hydroxy-3-indoleacetic acid (5-HIAA) are eluted together. The concentration of these metabolites is photometrical determined by the products formed in the reaction with 1-nitroso-2-naphthol.

## REAGENTS AND COLUMNS

Kit components:

<b>REAGENT 1</b> Hydrochloric acid	<b>1 x 95 ml</b>
<b>*REAGENT 2</b> Hydrated ammonium	<b>1 x 40 ml</b>
<b>*REAGENT 3</b> Diluted sulphuric acid	<b>1 x 25 ml</b>
<b>*REAGENT 4</b> 1-nitroso-2-naphthol	<b>1 x 25 ml</b>
<b>REAGENT 5</b> Sodium nitrite	<b>1 x 2 ml</b>
<b>*REAGENT 6</b> 1,2-dichloroethane	<b>1 x 65 ml</b>
<b>STANDARD</b> Serotonin 20 mg/L	<b>1 x 5 ml</b>
<b>COLUMNS</b> Chromatographic columns	<b>20</b>

(\*) Dangerous reagents are marked by an asterisk. Refer to MSDS.

STABILITY: stored at 2-8°C, sealed reagents and columns, are stable up to expiration date on the label.

## EQUIPMENT REQUIRED BUT NOT SUPPLIED

Spectrophotometer or filter photometer (540 nm), centrifuge.

## SAMPLE

24 hour urine.

Collect the 24 hour urine in a container with 2-3 ml of concentrated hydrochloric acid. Make sure the pH value is lower than 6. Mix the urine, measure the volume and store at 2-8°C.

STABILITY: about 5 days at 2-8°C with pH lower than 6.

## MANUAL ASSAY PROCEDURE

Wavelength:	540 nm
Temperature:	room temperature
Method:	colorimetric endpoint
Linearity:	50 mg/L
Sensitivity:	0.1 mg/L
Recovery:	98±2 %
C.V. (intra-assay):	3 %
C.V. (inter-assay):	4 %

## PREPARATION OF THE COLUMN

Let the columns reach room temperature before use. Shake and turn the columns upside down to obtain a complete resin re-suspension and leave them for a few minutes in vertical position to allow the resin to sediment again. Take the upper cap off and snap the bottom tip off. Let the liquid completely flow.

## CHROMATOGRAPHIC SEPARATION

Let the reagents reach room temperature before use. Gently pipette into a chromatographic column:

Reagent 1	1.0 ml	discard the eluate
Urine	1.0 ml	discard the eluate

Gently pipette, washing any eventual urine remains on the column walls:

Reagent 1	1.0 ml	discard the eluate
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Pipette:

Reagent 1	2.5 ml	collect the ELUATE 1
Reagent 2	0.5 ml	discard the eluate
Reagent 2	1.0 ml	collect the ELUATE 2

Accurately mix each collected eluate, keeping them separate.

## COLORIMETRIC REACTION

Pipette into centrifuge test-tubes:

	Sample		Standard	Blank
	5HT+5HTP	5-HIAA		
ELUATE 1	1.0 ml	---	---	---
ELUATE 2	---	1.0 ml	---	---
Standard	---	---	0.25 ml	---
Reagent 2	---	---	0.75 ml	1.0 ml
Reagent 3	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Reagent 5	1 drop (25 µl)	1 drop (25 µl)	1 drop (25 µl)	1 drop (25 µl)

Mix very carefully and wait for 10 minutes at room temperature.

Then pipette:

Reagent 6	1.5 ml	1.5 ml	1.5 ml	1.5 ml
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Shake on vortex for 10 seconds and centrifuge for 2 minutes at 3000 rpm. Read the absorbencies of the sample (As) and the standard (Astd), at 540 nm (510-570 nm) against blank. **Read in 1 ml Cuvette.**

## CALCULATION

### 5-HIAA

5-HIAA (mg/L) = (As / Astd) x 5.5

### 5HT + 5HTP

Serotonin (mg/L) = (As/ Astd) x 12.5

To calculate the 24 hour excretion, multiply the obtained values by the urine volume expressed in liters.

## REFERENCE VALUES

5-HIAA: 2 – 10 mg/24 hours

5HT + 5HTP: 0 – 5 mg/24 hours

## NOTES

- Aspirin, phenacetine, acetanilide, chlorpromazine give positive interference, same as drugs like reserpine which causes serotonin release. Phenothiazine derivatives inhibit the reaction. Do not administer any of these drugs for 72 hours before urine collection. For the same period of time, ban bananas, walnuts and tomatoes from the diet as their high concentration of tryptophane may give positive interference.
- If the supernatant is not completely clear after centrifuge, transfer it into another centrifuge test-tube containing a spatula-tip of anhydrous sodium sulfate, mix thoroughly and centrifuge or let deposit.
- In case of thrombosis and hemorrhage, 5-HIAA values ranged among 10 and 100 mg/24 hours may result; they are higher than 100 mg/24 hours (up to 2000 mg) in cases of argentaffinoma or malignant carcinoids. Patients with non-metastasing tumours may have normal values. In cases of pulmonary carcinomas, values are lightly increased. This determination is very useful in preliminary tests of suspected cases; no false positive values have been referred so far.
- We recommend using 1 ml glass/quartz cuvettes.
- Define ELUATE I and ELUATE II each time. 5-HIAA considerably increases in most cases of metastasing tumours, while sometimes only 5HT and 5HTP considerably increase. In such cases the test result will always be positive and values higher than normal values.

## REFERENCE

1. B.L. Goodwin, C.R.J. Ruthven, M.W. Weg and M. Sandler - Clin. Chim. Acta, 62 (1975) –439-442.



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