

HIPPURIC ACID

Colorimetric determination
of Hippuric Acid and Methyl-Hippuric Acid in Urine

100 tests

REF CM01-100T

INTENDED USE

Kit for quantitative *in vitro* determination of Hippuric Acid and Methyl-Hippuric Acid on urine.

PRINCIPLE

Hippuric acid, meta- and para-methyl hippuric acid form a colored complex with benzensulfonilchloride in pyridine, which may be determined photometrically.

REAGENTS

Kit components:

*REAGENT 1 Pyridine

REF CM01-100T
CM01-100TR1: 1 x 27 ml

*REAGENT 2 Benzensulfonilchloride

CM01-100TR2: 1 x 13 ml

STANDARD Hippuric Acid Standard 500 mg/L

CM01-100TS: 1 x 3 ml

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

STABILITY: stored at 4-25°C, sealed reagents are stable up to the expiration date on the label.

REQUIRED REAGENTS BUT NOT PROVIDED

95% ethylic alcohol
Chloroform

REQUIRED EQUIPMENT

Centrifuge, spectrophotometer or filter photometer at 410 nm (400 - 440 nm).

SAMPLE

24-hour urine.

Collect the 24-hour urine in a container containing 4-5 ml chloroform. Centrifuge 2-3 ml urine at 3000 rpm for 5 minutes.

STABILITY: at least one week at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength: 410 nm (400 – 440 nm)
Optical path: 1 cm
Reading: against 95% ethanol
Temperature: room temperature
Method: colorimetric endpoint
Linearity: up to 4 g/L
Minimum sensitivity: 0.1 g/L
C.V. (intra-assay): 6 %

Pipette into 2 centrifuge test tubes labeled as follows:

	Sample	Standard
Sample	0.25 mL	---
Standard	---	0.25 mL
Reagent 1	0.25 mL	0.25 mL
Reagent 2	0.10 mL	0.10 mL

Mix well. Let stand for 30 minutes, then add:

95% ethylic alcohol:	5.0 mL	5.0 mL
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Mix well, centrifuge for 5 minutes at 2500-3000 rpm and read the optical densities of the sample and of the standard against 95% ethanol.

CALCULATION

Hippuric acid (mg/L) = (A sample / A standard) x 500

mg hippuric acid/liter x L.s of urine/24h= mg hippuric acid/24h

NOTE: With 'hippuric acid' we mean the addition of hippuric and methyl-hippuric acids, as the kit cannot distinguish them.

REFERENCE VALUES

200 -1600 mg/24 hours.

PERFORMANCE CHARACTERISTICS

Sensitivity: the sensitivity of the method is 100 mg/L.

Linearity: up to 4 g/L.

For higher values, dilute the sample 1:10 with saline solution and multiply the result by 10.

Precision:

Within run (n=10)	Mean [U/L]	CV %
Sample 1	300	6.2
Sample 2	1400	6.0

Between run (n=20)	Mean [U/L]	CV %
Sample 1	250	4.2
Sample 2	1400	6.2

Correlation against a reference method: the correlation of FAR method (Y) against a reference method (X) gives a correlation of 0,9956

DISPOSAL

The product must be used for professional assay only. Dispose of the product according to national/international laws.

WARNINGS AND PRECAUTIONS



REAGENT 1

H225

Highly flammable liquid and vapor.

H302+H312+H332

Harmful if swallowed, in contact with skin, if inhaled.

H315

Causes skin irritation.

H319

Causes serious eyes irritation.



REAGENT 2

H302

Harmful if swallowed.

H314

Causes severe skin burns and eyes damage.

H317

May cause allergic skin reaction.

H332

Harmful if inhaled.

H334

May cause allergy or asthma symptoms or breathing difficulties if inhaled.

STANDARD: Non dangerous.

NOTES

- High levels of hippuric acid in urine indicate that the patient has been exposed to toluene or xylene fumes. These solvents are often used in industrial processes.
- False high results can be caused by food preservatives containing benzoate, salicylic and acetylsalicylic acid. The patient should avoid all food containing these preservatives three days before urine collection.
- Normal values for hippuric acid can reach 2000 mg/liter in a single voided urine sample. It is absolutely necessary to take the sample from the entire volume of thoroughly mixed 24-hour urine.
- Pyridine and benzensulfonilchloride must be dispensed with an automatic pipette or a pipette aspirator. Keep the vials tight closed.
- A proportional variation in reagent volumes does not change the results.

KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	read instructions for use

IVD

CE

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MANUFACTURER



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