

# 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-ippuric 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

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

**IVD**

**CE**

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## MANUFACTURER



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