# Uric acid

Uricase -POD. Enzymatic colorimetric

Quantitative determination of uric acid

CE IVD

***PACKAGING***

| | |
|---|---|
| Ref.: 101-0332 | Cont.: 4 x 50 mL |
| Ref.: 101-0324 | Cont.: 5 x 20 mL |

Store at 2 - 8º C

***CLINICAL SIGNIFICANCE***

Uric acid and its salts are end products of the purine metabolism.

With progressive renal insufficiency, there is retention in blood of urea, creatinine and uric acid. Elevate uric acid level may be indicative of renal insufficiency and is commonly associated with gout[1,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

***PRINCIPLE OF THE METHOD***

Uric acid is oxidized by uricase to allantoine and hydrogen peroxide ($2H_2O_2$), which under the influence of POD, 4–aminophenazone (4-AP) and 2-4 Dichlorophenol sulfonate (DCPS) forms a red quinoneimine compound:

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + 2H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{DCPS} \xrightarrow{\text{POD}} \text{Quinoneimine} + 4H_2O$$

The intensity of the red color formed is proportional to the uric acid concentration in the sample[1,2].

***REAGENTS***

| | | |
|---|---|---|
| **R 1** Buffer | Phosphate pH 7.4 | 50 mmol/L |
| | 2-4 Dichlorophenol sulfonate (DCPS) | 4 mmol/L |
| **R 2** Enzymes | Uricase | 60 U/L |
| | Peroxidase (POD) | 660 U/L |
| | Ascorbate oxidase | 200 U/L |
| | 4 – Aminophenazone (4-AP) | 1 mmol/L |
| **URIC ACID CAL** | Uric acid aqueous primary standard 6 mg/dL | |

**Optional (not included in the kit)**

| | | | |
|---|---|---|---|
| Contro-N | Ref.: 101-0252 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0083 | 20 x 5 mL | |
| Contro-P | Ref.: 101-0253 | 4 x 5 mL | Lyophilized human control serum |
| | Ref.: 101-0084 | 20 x 5 mL | |

***PREPARATION***

Working reagent (WR): Dissolve the contents of one vial R 2 Enzymes in one bottle R 1 Buffer. Cap and mix gently to dissolve contents. (WR) is stable after reconstitution 1 month at 2 - 8º C or 10 days at room temperature.

***STORAGE AND STABILITY***

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

**Signs of reagent deterioration:**

- Presence of particles and turbidity.
- Blank absorbance (A) at 520 nm ≥ 0.16.

***ADDITIONAL EQUIPMENT***

- Spectrophotometer or colorimeter measuring at 520 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

***SAMPLES***

- Serum or plasma[1]: Stability 3 - 5 days at 2 - 8º C or 6 months at –20º C.
- Urine (24 h)[1]: Stability 4 days at 15 - 25º C, pH >8. Dilute sample 1/50 in distilled water. Mix. Multiply results by 50 (dilution factor);
  If urine is cloudy; warm the specimen to 60º C for 10 min to dissolve precipitated urates and uric acid. Do not refrigerate.

***PROCEDURE***

**Notes:** CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

URIC ACID CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.

Use clean disposable pipette tips for its dispensation.

1. Assay conditions:
   Wavelength: . . . . . .520 nm (490 - 550)
   Cuvette: . . . . . 1 cm light path
   Temperature . . . . . 37º C / 15 - 25º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

| | Blank | Standard | Sample |
|---|---|---|---|
| WR (mL) | 1.0 | 1.0 | 1.0 |
| Standard[(Note 1-2)] (µL) | -- | 25 | -- |
| Sample (µL) | -- | -- | 25 |

4. Mix and incubate for 5 min at 37º C or 10 min at 15 - 25º C.
5. Read the absorbance (A) of the samples and Standard, against the Blank. The colour is stable for at least 30 minutes.

***CALCULATIONS***

Serum or plasma

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 6 \text{ (Standard conc.)} = \text{mg/dL uric acid in the sample}$$

Urine 24 h

$$\frac{(A)\text{Sample}}{(A)\text{Standard}} \times 6 \times \text{vol. (dL) urine 24 h} = \text{mg/24 h uric acid}$$

**Conversion factor:** mg/dL x 59.5= µmol/L.

***QUALITY CONTROL***

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

***REFERENCE VALUES[5]***

Serum or plasma:

| | | | |
|---|---|---|---|
| Women | 2.5 - 6.8 mg/dL | ≅ | 149 – 405 µmol/L |
| Men | 3.6 - 7.7 mg/dL | ≅ | 214 – 458 µmol/L |
| Urine: | 250 - 750 mg/24 h | ≅ | 1.49 - 4.5 mmol/24 h |

These values are for orientation purpose; each laboratory should establish its own reference range.



CHRONOLAB SYSTEMS, S.L., Travessia Prat de la Riba 34 B, 08849 Sant Climent de Llobregat, Barcelona, Spain
Tel. +34 617722466, www.chronolab.com, e-mail: info@chronolab.com

***PERFORMANCE CHARACTERISTICS***

**Measuring range:** From detection limit of 0.03 mg/dL to linearity limit of 25 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

**Precision:**

| | Intra-assay (n=20) | | Inter-assay (n=20) | |
|---|---|---|---|---|
| Mean (mg/L) | 4.74 | 11.4 | 4.72 | 11.2 |
| SD | 0.03 | 0.06 | 0.07 | 0.15 |
| CV (%) | 0.63 | 0.56 | 1.58 | 1.36 |

**Sensitivity:** 1 mg/dL = 0.0347 A.

**Accuracy:** Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).
The results obtained using 50 samples were the following:
Correlation coefficient (r): 0.99.
Regression equation: y=1.005x + 0.0005.
The results of the performance characteristics depend on the analyzer used.

***INTERFERENCES***

No interferences were observed to bilirubin up to 170 µmol/L, hemoglobin up to 130 mg/dL and ascorbic acid up to 570 µmol/L[2].
A list of drugs and other interfering substances with uric acid determination has been reported by Young et. al[3,4].

***BIBLIOGRAPHY***

1. Schultz A. Uric acid. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1261-1266 and 418.
2. Fossati P et al. Clin Chem 1980;26:227-231.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
6. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.


CHRONOLAB SYSTEMS, S.L., Travessia Prat de la Riba 34 B, 08849 Sant Climent de Llobregat, Barcelona, Spain
Tel. +34 617722466, www.chronolab.com, e-mail: info@chronolab.com