ISE S.r.l. **Uric Acid** – Instructions for use

CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

CE IVD

R1: 2x 18 mL R2: 2x 4,5 mL **R3330000027** REF

Diagnostic reagent for quantitative in vitro determination of uric acid in human serum, plasma or urine on photometric systems

test parameters

Method:	Colorimetric, enzymatic, endpoint, increasing reaction
Wavelength:	520 nm, Hg 546 nm (500 – 550 nm)
Temperature:	20 – 25 °C, 37 °C
Sample:	Serum, heparin or EDTA plasma, urine
Linearity:	up to 20 mg/dL (1190 µmol/L)
Sensitivity:	The lower limit of detection is 0.07 mg/dL (4.2 µmol/L).

SUMMARY [1,2]

Uric acid and its salts are end products of the purine metabolism. In gout, the most common complication of hyperuricemia, increased serum levels of uric acid lead to formation of monosodium urate crystals around the joints. Further causes of elevated blood concentrations of uric acid are renal diseases with decreased excretion of waste products, starvation, drug abuse and increased alcohol consume as well as use of certain medicaments. High uric acid levels also constitute an indirect risk factor for coronary heart disease. Hypouricemia is seldom observed and associated with rare hereditary metabolic disorders.

TEST PRINCIPLE

Uric acid is oxidized to allantoin by uricase. The generated hydrogen peroxide reacts with 4-aminoantipyrine and 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) to quinoneimine.

$$\text{Uric Acid} + H_2O + O_2 \xrightarrow{\text{URICASE}} \text{Allantoin} + CO_2 + H_2O_2$$

$$\text{TBHBA} + \text{4-AAP} + H_2O_2 \xrightarrow{\text{POD}} \text{Quinoneimine} + 3\,H_2O$$

abbreviations

4-AAP	=	4-Aminoantipyrine
POD	=	Peroxidase
TBHBA	=	2,4,6-Tribromo-3-hydroxybenzoic acid

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent 1:		
Phosphate Buffer, pH 7.0	100	mmol/L
TBHBA	1.25	mmol/L
Reagent 2:		
Phosphate Buffer, pH 7.0	100	mmol/L
4-Aminoantipyrine	1.5	mmol/L
K_4 [Fe(CN)$_6$]	50	µmol/L
POD	≥ 10	kU/L
Uricase	≥ 150	U/L

REAGENT PREPARATION

Substrate Start
The reagents are ready to use.
Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= Working Reagent)

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light. Close immediately after use. Do not freeze the reagents! Avoid contamination.	
Substrate Start:		
Stability:	at 2 – 8 °C	up to the expiration date
Sample Start (Working Reagent):		
Stability:	at 2 - 8 °C	3 months
	at 15 – 25 °C	2 weeks

Protect the Working Reagent from light!

Note: The measurement is not influenced by occasionally occurring colour changes, as long as the absorbance of the Working Reagent is < 0.5 at 546 nm.

sample PREPARATION

Urine: Dilute urine 1 + 10 with dist. water.

SAMPLE STABILITY AND STORAGE

serum/plasma [3]:	at 20 – 25 °C	3 days
	at 4 – 8 °C	7 days
	at -20 °C	6 months
Urine [4]:	at 20 – 25 °C	4 days

Freeze only once! Discard contaminated specimens.

materials required but not provided

NaCl solution (9 g/L)
General laboratory equipment

STandard

(not included in the kits – has to be ordered separately)

Concentration	6 mg/dL (357 µmol/L)
Storage:	2 – 8 °C
Stability:	up to the expiration date

Close immediately after use! Avoid contamination!

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Substrate Start

Pipette into test tubes	Blank	Std./Cal.	Sample
Reagent 1	1000 µL	1000 µL	1000 µL
Sample or Std./Cal.	-	20 µL	20 µL
Distilled water	20 µL	-	-
Mix. Incubate 5 min. at 20 – 25 °C / 37 °C. Then add:			
Reagent 2	250 µL	250 µL	250 µL
Mix. Incubate 30 min. at 20 – 25 °C or 10 min. at 37°C. Measure absorbance of sample and std./cal. against reagent blank within 60 minutes.			

Sample Start

Pipette into test tubes	Blank	Std./Cal.	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Sample or Std./Cal.	-	20 µL	20 µL
Distilled water	20 µL	-	-
Mix. Incubate 30 min. at 20 – 25 °C or 10 min. at 37 °C. Measure absorbance of sample and std./cal. against reagent blank within 60 minutes.			

CALCULATION

Serum/Plasma:

$$\text{Uric Acid [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]}$$

Urine:

$$\text{Uric Acid [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]} \times 11$$

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unit conversion

mg/dL x 59.48 = µmol/L

REFERENCE RANGE *

Serum/Plasma:

	Females		Males	
	mg/dL	mmol/L	mg/dL	mmol/L
Adults [5]	2.6 – 6.0	155 – 357	3.5 – 7.2	208 – 428

	Females		Males	
Children [6]	mg/dL	mmol/L	mg/dL	mmol/L
0 – 30 days	1.0 – 4.6	59 – 271	1.2 – 7.2	71 – 230
31 – 365 days	1.0 – 5.4	65 - 319	1.2 – 5.6	71 - 330
1 – 3 years	1.8 – 5.0	106 – 295	2.1 – 5.6	124 – 330
4 – 6 years	2.0 – 5.1	118 - 301	1.8 – 5.5	106 - 325
7 – 9 years	1.8 – 5.5	106 – 325	1.8 – 5.4	106 – 319
10 – 12 years	2.5 – 5.9	148 - 348	2.2 – 5.8	130 - 342
13 – 15 years	2.2 – 6.4	130 – 378	3.1 – 7.0	183 – 413
16 – 18 years	2.4 – 6.6	142 – 389	2.1 – 7.1	124 – 448

Urine [1]

assuming normal diet	≤ 800 mg/24h (4.76 mmol/24h)
assuming low purine diet	≤ 600 mg/24h (3.57 mmol/24h)

* Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

PERFORMANCE CHARACTERISTICS

LINEARITY / MEASURING RANGE

The test has been developed to determine uric acid concentrations within a measuring range from 0.07 – 20 mg/dL (4.2 – 1190 µmol/L). When values exceed this range, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and reassayed multiplying the result by 2.

SENSITIVITY/LIMIT OF DETECTION
The lower limit of detection is 0.07 mg/dL (4.2 µmol/L).

PRECISION (at 37°C)

Intra-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	2.75	0.04	1.55
Sample 2	5.35	0.04	0.74
Sample 3	10.1	0.08	0.77

Inter-assay, n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	2.68	0.04	1.52
Sample 2	5.23	0.09	1.63
Sample 3	9.98	0.11	1.06

SPECIFICITY/INTERFERENCES
no interference up to:

Bilirubin	10 mg/dL
Triglyceride	2000 mg/dL
Hemoglobin	100 mg/dL

Ascorbic acid interferes even in minimal concentrations.
For measurement without interference by ascorbic acid, we recommend the use of DIALAB Uric Acid AOX Reagent.
For further information on interfering substances refer to Young DS [7].

METHOD COMPARISON

A comparison between this Uric acid TBHBA (y) and a commercially available test (x) using 70 samples gave following results: y = 1.02 x – 0.44 mg/dl; r= 0.997.

calibration

The assay requires the use of a uric acid standard or calibrator.

QUALITY CONTROL

All controls with Uric Acid values determined by this method can be used.
Each laboratory should establish corrective action in case of deviations in control recovery.

WARNINGS AND PRECAUTIONS

1. Reagent 2 contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
2. In very rare cases, samples of patients with gammopathy might give falsified results [8].
3. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
5. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

WASTE MANAGEMENT

Please refer to local legal requirements.

References

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Symbols on labels and packaging

IVD = In vitro diagnostic medical device

REF = Catalog Number

LOT = Lot Number

= Manufacturer

= Expiration date

= Temperature limitation

= Instruction for use

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