Uric acid FS*

TBHBA

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

Order Information

Cat. No.	Kit size						
1 3021 99 10 021	R1	5 x	20 mL	+	R2	1 x	25 mL
1 3021 99 10 026	R1	5 x	80 mL	+	R2	1 x	100 mL
1 3021 99 10 023	R1	1 x	800 mL	+	R2	1 x	200 mL
1 3021 99 10 704	R1	8 x	50 mL	+	R2	8 x	12.5 mL

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 a indirect risk factor for coronary heart disease. Hypouricemia is seldom observed and associated with rare hereditary metabolic disorders.

Method

Enzymatic photometric test using TBHBA (2,4,6-Tribromo-3-hydroxybenzoic acid)

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.

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

$$\text{TBHBA} + \text{4-Aminoantipyrine} + 2\ H_2O_2 \xrightarrow{\text{POD}} \text{Quinoneimine} + 3\ H_2O$$

Reagents

Components and Concentrations

R1:	Phosphate buffer	pH 7.0	100 mmol/L
	TBHBA (2,4,6-Tribromo-3-hydroxybenzoic acid)		1.25 mmol/L
R2:	Phosphate buffer	pH 7.0	100 mmol/L
	4-Aminoantipyrine		1.5 mmol/L
	$K_4[Fe(CN)_6]$		50 µmol/L
	Peroxidase (POD)		≥ 10 kU/L
	Uricase		≥ 150 U/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Note: It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the monoreagent is < 0.5 at 546 nm.

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, metamizole and phenindione 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. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma, urine

Stability in serum/plasma [3]:

6 months	at	–20°C
7 days	at	4 – 8°C
3 days	at	20 – 25°C

Freeze only once.
Discard contaminated specimens.

Stability in urine [4]:

4 days	at	20 – 25°C

Dilute urine 1 +10 with dist. water and multiply the results by 11.
Discard contaminated specimens.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	520 nm, Hg 546 nm, 500 - 550 nm
Optical path	1 cm
Temperature	20 – 25°C/ 37°C
Measurement	Against reagent blank

	Blank	Sample/Calibrator
Sample/Calibrator	-	20 µL
Dist. water	20 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate 5 min., then add:		
Reagent 2	250 µL	250 µL
Mix, incubate 30 min. at 20 – 25°C or 10 min. at 37°C.		
Read the absorbance against the reagent blank within 60 min.		

Calculation

With calibrator

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

Conversion factor

Uric acid [mg/dL] x 59.48= Uric acid [µmol/L]

*fluid stable

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The assigned values of the calibrator have been made traceable to the reference method gas chromatography-isotope dilution mass spectrometry (GC-IDMS). Uric acid Standard FS may be used alternatively for calibration. DiaSys TruLab N and P controls should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL
TruLab Urine Level 1	5 9170 99 10 062	20 x 5 mL
	5 9170 99 10 061	6 x 5 mL
TruLab Urine Level 2	5 9180 99 10 062	20 x 5 mL
	5 9180 99 10 061	6 x 5 mL
Uric acid Standard FS	1 3000 99 10 030	6 x 3 mL

Performance Characteristics

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 the result multiplied by 2.

Specificity/Interferences

No interference was observed by bilirubin up to 10 mg/dL and lipemia up to 2000 mg/dL triglycerides. Hemoglobin interferes starting with a concentration of 100 mg/dL. Ascorbic acid interferes even in minimal concentrations.
For measurement without interference by ascorbic acid DiaSys Uric acid FS TOOS is recommended. For further information on interfering substances, refer to Young DS [7].

Sensitivity/Limit of Detection

The lower limit of detection is 0.07 mg/dL.

Precision (at 37°C)

Intra-assay precision 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 precision 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

Method Comparison

A comparison of DiaSys Uric acid FS TBHBA (y) with a commercially available test (x) using 70 samples gave the following results: y = 1.02 x – 0.44 mg/dL; r= 0.997

Reference Range

Serum/Plasma

	Female mg/dL (µmol/L)	Male mg/dL (µmol/L)
Adults [5]	2.6 – 6.0 (155 – 357)	3.5 – 7.2 (208 – 428)
Children [6]		
1 – 30 days	1.0 – 4.6 (59 – 271)	1.2 – 3.9 (71 – 230)
31 – 365 days	1.1 – 5.4 (65 – 319)	1.2 – 5.6 (71 – 330)
1 – 3 year(s)	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 – 7.6 (124 – 448)

Urine [1]

≤ 800 mg/24h (4.76 mmol/24h) assuming normal diet

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

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

Literature

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 208-14.
2. Newman DJ, Price CP. Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1204-70.
3. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 48-9.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 52-3.
5. Newman JD, Price PC. Renal function and nitrogen metabolites. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1250.
6. Soldin SJ, Brugnara C, Wong EC. Pediatric Reference Intervals, 6th ed. Washington DC; The American Association for Clinical Chemistry Press, 2007; p. 204-5
7. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45(9):1240–1243.

Manufacturer

IVD CE

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