

Liquid Reagents – ready to use

URIC ACID TBHBA

Enzymatic, Colorimetric with ATCS*
 2 Reagents

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

REF	Kit Size	Content
D95458B	1 x 12.5 L	1 x 10 L R1 + 2.5 L R2
D03123B	1 x 1.25 L	1 x 1 L R1 + 1 x 250 mL R2
D95459	5 x 100 mL	4 x 100 mL R1 + 1 x 100 mL R2
D98461	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
D00463	5 x 25 mL	4 x 25 mL R1 + 1 x 25 mL R2
D00464	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
D83911	10 x 50 mL	10 x 40 mL R1 + 4 x 25 mL R2
D0441917	5 x 62.5 mL	4 x 62.5 mL R1 + 1 x 62.5 mL R2
DA0847	5 x 50 mL	5 x 40 mL R1 + 5 x 10 mL R2
DT1047	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2
DK0744	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DB0947	2 x 150 mL	2 x 120 mL R1 + 2 x 30 mL R2

Additionally offered:

D94708	1 x 3 mL	Uric Acid Standard	
D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P
D08581	12 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08581SV	1 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08582	12 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2
D08582SV	1 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2

* Advanced Turbidity Clearing System; minimizes turbidity caused by lipemia

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.



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:

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

UNIT CONVERSION

$$\text{mg/dL} \times 59.48 = \mu\text{mol/L}$$

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	
	mg/dL	mmol/L	mg/dL	mmol/L
Children [6]				
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 Dialab Uric acid TBHBA (y) and a commercially available test (x) using 70 samples gave following results: $y = 1.02x - 0.44$ mg/dl; $r = 0.997$.

CALIBRATION

The assay requires the use of a uric acid standard or calibrator. We recommend the Dialab **Uric Acid Standard** and the Dialab multi calibration serum **Diacal Auto**.

Calibrator values have been made traceable to the reference method gas chromatography – isotope dilution mass spectrometry (GC-IDMS).

QUALITY CONTROL

All controls with Uric Acid values determined by this method can be used.

We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range) as well as the Dialab urine controls **Diacon Urine Level 1** (control urine normal) and **Level 2** (control urine abnormal).

Each laboratory should establish corrective action in case of deviations in control recovery.

AUTOMATION

Applications for automated systems are available upon request.

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

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. ClinChemLabMed 2007; 45(9): 1240-1243.

