Instructions for Use

For in vitro diagnostic use

ÁCIDO ÚRICO WS

URIC ACID WS

Anvisa 80115310194

ORDER INFORMATION

Cat. No.	Kit size
1010250KWS	R1 1x200mL + R2 1x50mL
1010100MKWS	R1 2x40mL + R2 2x10mL
1010500KWS	R1 2x200mL + R2 1x100mL
1010150MWS	R1 4x30mL + R2 2x15mL
1010200RWS	R1 4x38,6mL + R2 4x11,4mL

INTENDED USE

Diagnostic reagent for quantitative *in vitro* determination of Uric Acid in serum, plasma or urine on photometric systems

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, the colorimetric indicator.

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

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

REAGENTS

Components and Concentrations

R1	Sodium dihydrogen phosphate monohydrate	0.1 mol/L
	TBHBA (2,4,6-tribromo-3-hydroxybenzoic acid)	<3 mmol/L
R2	Sodium dihydrogen phosphate monohydrate	0.1 mol/L
	4-Aminoantipyrine	<2.0 mmol/L
	$K_4[Fe(CN)_6]$	0.05 mmol/L
	Peroxidase (POD)	<50 kU/L
	Uricase	<300 U/L

STORAGE AND 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

Follow the requirements of the current guidelines about technical regulation for the management of healthcare service waste, as well as other equivalent biosafety practices.

REAGENT PREPARATION

Starting with Substrate

The reagents are ready to use.

Starting with Sample

Mix 4 parts of R1 with 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = monoreagent

Stability: 3 months at 2 - 8 ºC
2 weeks at 15 - 25 ºC

Protect the monoreagent from light!

MATERIALS REQUIRED, BUT NOT PROVIDED

1. NaCl solution 9 g/L.
2. General laboratory equipment.

SPECIMEN

Serum, heparin plasma or EDTA plasma, urine

Stability in serum/plasma [3]: 3 days at 20 - 25 ºC
7 days at 4 - 8 ºC
6 months at -20 ºC

Freeze only once.

Discard contaminated specimens.

Stability in urine [4]: 4 days at 20 - 25 ºC

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

ASSAY PROCEDURE

Applications for automatic systems are available upon request or on our website: www.kovalent.com.br

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

Starting with Substrate

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

Starting with Sample

	Blank	Sample or standard
Sample or calibrator	-	20 µL
Distilled water	20 µL	-
Monoreagent	1000 µL	1000 µL
Mix, incubate for 30 minutes at 20 – 25°C or 10 minutes at 37°C. Read the absorbance against the reagent blank within 60 minutes.		

CALCULATION

With calibrator

$$\text{Uric acid } \left[\frac{\text{mg}}{\text{dL}}\right] = \frac{\Delta A\ Sample}{\Delta A\ Calibrator}\ x\ Conc.calibrator\ \left[\frac{mg}{dL}\right]$$

Conversion factor

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

CALIBRATORS AND CONTROLS

For calibration in automated photometric systems, Kovalent Topkal U calibrator is recommended. Use Kovalent Topkon N and P for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

WARRANTY

These instructions for use should be read carefully before using the product and the information contained therein should be strictly adhered to. The reliability of the test results cannot be guaranteed if the instructions are not followed.

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 of triglycerides. Hemoglobin interferes starting with a concentration of 100 mg/dL. Ascorbic acid interferes even in minimal concentrations. 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 (4.2 µmol/L).

Precision

Intra-assay precision n = 10	Mean [mg/dL]	SD [mg/dL]	CV [%]
Controle normal	6.61	0.03	0.44
Controle patológico	9.48	0.04	0.46

Inter-assay precision n = 9	Mean [mg/dL]	SD [mg/dL]	CV [%]
Controle normal	6.64	0.11	1.66
Controle patológico	9.48	0.05	0.52

Method comparison

A comparison of Kovalent Ácido Úrico WS (y) with a commercially available test (x) using 30 samples gave the following results:
$y = 1.0314\ x - 0.2938$; $R^2 = 0.9981$

REFERENCE VALUES

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 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 – 7.6 (124 – 448)

Urine

≤ 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. Neweman DJ, Price CP. Renal function and nitrogen metabolites. In.: Burtis CA, Ashwood ER, editors. Tietz Textsbook of Clinical Chemitry. 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.
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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, Mucke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45(9):1240-1243.

CONSUMER INFORMATION

Symbols used:

	Manufacturer
	Temperature limit
	In vitro diagnostic device
	Caution
	Operating instructions
	Recycling material
	Do not discard directly into the environment
	Batch code
	Date of manufacture
	Use by date
	Biological hazards
	Highly toxic
	Corrosive
	Harmful

Manufacturer:

Kovalent do Brasil Ltda.
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São Gonçalo – RJ – CEP 24722-414 - Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit size variations on demand:

Anvisa No.	Kit size
80115310194	R1 4x40mL + R2 4x10mL
80115310194	R1 1x40mL + R2 1x10 mL
80115310194	R1 3x40mL + R2 3x10 mL

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Expiration date and Lot no.: See label