Instructions for Use
For *in vitro* diagnostic use

CREATININA WS

Creatinine WS

Anvisa 80115310205

ORDER INFORMATION

Cat. No.	Kit size
1030500KWS	R1 2 x 200 mL + R2 1 x 100 mL
1030150MWS	R1 4 x 30 mL + R2 2 x 15 mL
1030200RWS	R1 4 x 38,6 mL + R2 4 x 11,4 mL
1030100MKWS	R1 2 x 40 mL + R2 2 x 10 mL
1030250KWS	R1 1 x 200 mL + R2 1 x 50 mL

INTENDED USE

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

SUMMARY

Creatinine is a waste product excreted by the kidneys mainly by glomerular filtration. The concentration of creatinine in plasma of a healthy individual is fairly constant, independent from water intake, exercise and rate of urine production. Therefore, increased plasma creatinine values always indicate decreased excretion, i.e. impaired kidney function. Creatinine clearance is a good indicator for the glomerular filtration rate (GFR) which allows better detection of kidney diseases and monitoring of renal function. For this purpose, creatinine is measured simultaneously in serum and urine collected over a defined time period. [1,2]

METHOD

Kinetic test without deproteinization according to the Jaffé method

PRINCIPLE

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid ———► Creatinine picrate complex

REAGENTS

Components and Concentrations

R1	Sodium hydroxide	0.2 mol/L
R2	Picric acid	20 mmol/L

WARNINGS AND PRECAUTIONS

1. Reagent 1: Warning! May be corrosive to metals. It causes skin irritation. Causes serious eye irritation, if irritation persists, seek medical advice. Keep only in original packaging. Wash hands and face thoroughly after handling. Wear protective gloves/protective clothing/eye protection. IF ON SKIN: Wash with plenty of water/soap. If skin irritation occurs: Get medical advice/attention. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do, and continue rising. Absorb spillage to prevent material damage
2. Reagent 2: Warning! May be corrosive to metals. Keep only in original packaging. Wear protective gloves/protective clothing/eye protection. Absorb spillage to prevent material damage.
3. High homogentisic acid concentrations in urine samples lead to false results.
4. In very rare cases, samples of patients with gammopathy might give falsified results [11].
5. Eltrombopag medication (active ingredient of the drug Revolade®) leads to falsely low or high results in patient samples.
6. Please refer to the safety data sheets (SDS) 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.
7. For professional use only.

STORAGE AND STABILITY

Reagents are stable up to the date of expiry indicated on the kit, if stored at 2 - 8°C, protected from light and if contamination is avoided. Do not freeze the reagents!

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: 5 hours at 15-25°C

MATERIALS REQUIRED BUT NOT PROVIDED

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

SPECIMEN

Serum, heparin plasma or urine

Stability [5]:

Serum/plasma:	7 days	at	4 - 25 ºC
	at least 3 months	at	-20 ºC
Urine:	2 days	at	20 - 25 ºC
	6 days	at	4 - 8 ºC
	6 months	at	-20 ºC

Dilute urine 1 + 49 with distilled water; multiply the result by 50.

Discard contaminated specimens! Only freeze once!

ASSAY PROCEDURE

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

Wavelength	Hg 492nm (490 - 510 nm)
Optical path	1 cm
Temperature	20 - 25 ºC / 37 ºC
Measurement	Against reagent blank

Starting with Substrate

	Blank	Sample or calibrator
Sample or calibrator	-	50 µL
Distilled water	50 µL	-
Reagent 1	1000 µL	1000 µL
Mix, incubate for 0 - 5 min, then add:		
Reagent 2	250 µL	250 µL
Mix and read the absorbance A1 after 60 seconds, then read the absorbance A2 after the next 120 seconds.		

$$\Delta A = (A2 - A1)_{\text{sample or calibrator}}$$

Starting with Sample

	Blank	Sample or calibrator
Sample or calibrator	-	50 µL
Distilled water	50 µL	-
Monoreagent	1000 µL	1000 µL
Mix and read the absorbance A1 after 60 seconds, then read the absorbance A2 after the next 120 seconds.		

$$\Delta A = (A2 - A1)_{\text{sample or calibrator}}$$

CALCULATION

With calibrator

Serum/plasma

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

Urine

$$\text{Creatinine [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Cal.}} \times \text{Conc Cal.[mg/dL]} \times 50$$

Creatinine *Clearance* [mL/min/1.73 m²] [7]

$$= \frac{\text{mg Creatinine / 100 mL Urine x mL Urine}}{\text{mg Creatinine / 100 mL Serum x min (urine collection time)}}$$

The calculated creatinine clearance refers to the average body surface of an adult (1.73 m^2).

Conversion Factor

Creatinine [mg/dL] x 88.4 = Creatinine [µmol/L]

COMPENSATED METHOD [3,4]

Picric acid which forms the colored complex reacts unspecifically with interfering serum components, so-called pseudo-creatinines. This leads to falsely elevated creatinine values in serum and plasma samples especially in the low measuring range. To compensate these interferences, the calibrator value for the compensated method indicated in the value sheet of Topkal U has to be used for calculation. Additionally, 0.3 mg/dL has to be subtracted from the calculated creatinine value. For use of the compensated method, calibration with the calibrator Topkal U is strictly recommended. The method is applicable only for serum and plasma samples. The compensated method is traceable to GC-IDMS.

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 is designed to determine creatinine concentrations within a measuring range of 0.2 - 15 mg/dL (18 - 1330 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 ascorbic acid up to 30 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 2000 mg/dL of triglycerides. Bilirubins interfere from a concentration of 4 mg/dL. For more information on interfering substances, see Young DS [10].

Sensitivity / Limit of Detection
The lowest detection limit is 0.2 mg/dL (17.7 µmol/L).

Precision

Within run n = 10	Mean [mg/dL]	SD [mg/dL]	CV [%]
Normal control	1.44	0.04	3.13
Pathological control	4.089	0.06	1.50

Between day n = 9	Mean [mg/dL]	SD [mg/dL]	CV [%]
Normal control	1.51	0.02	1.22
Pathological control	4.26	0.08	1.93

Method comparison

Method comparison between Kovalent Creatinina (y) and a commercially available test with Jaffé method (x) using 30 samples of human serum demonstrated the following results:
$y = 0.9939x + 0.0067$; $R^2 = 0.9839$

REFERENCE RANGE

Serum or plasma – Jaffé method, not compensated

	mg/dL	**µmol/L**
Adults [1]		
Women	0.6 – 1.1	53 – 97
Men	0.7 – 1.3	62 – 115
Children [2,8]		
Neonate	0.5 – 1.2	44 – 106
Infant	0.4 – 0.7	35 – 62
Child	0.5 – 1.2	44 – 106

Serum or plasma – Jaffé method, compensated

	mg/dL	**µmol/L**
Adults [3]		
Women	0.5 – 0.9	44 – 80
Men	0.7 – 1.2	62 – 106
Children [9]		
Neonate	0.24 – 1.04	21 – 92
Infant	0.17 – 0.42	15 – 37
Child	0.24 – 0.87	21 – 77

1st urine of the morning [3] – Jaffé method, compensated

	mg/dL	**µmol/L**
Women	28 – 217	2470 – 19200
Men	39 - 259	3460 - 22900
	mg/kg/24 h	**µmol/kg/24 h**
Urine 24h [1]		
Women	11 – 20	97 – 177
Men	14 – 26	124 - 230

Albumin/creatinine ratio (early morning urine) [12]:
< 30 mg/g Creatinine

Creatinine *Clearance* [2]	**mL/min/1,73 m^2**
Women	95 – 160
Men	98 - 156

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

LITERATURE

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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.
Rua Cristóvão Sardinha, 110 – Jd. Bom Retiro
São Gonçalo – RJ – CEP 24722-414 – Brasil
www.kovalent.com.br
CNPJ: 04.842.199/0001-56

Kit sizes variations on demand:

Anvisa No.	Kit size
80115310205	R1 4x40mL + R2 4x10mL
80115310205	R1 1x40mL + R2 1x10mL
80115310205	R1 3x40mL + R2 3x10mL

Costumer service: sac@kovalent.com.br - (21) 3907-2534 / 0800 015 1414

Expiration date and Lot no.: See label