**Instructions for Use**
For *in vitro* diagnostic use

# UREIA UV WS

Anvisa 80115310189

### ORDER INFORMATION

| Cat. No. | Kit size |
|---|---|
| 1070500KWS | R1: 2 x 200 mL + R2: 1 x 100 mL |
| 1070150MWS | R1: 4 x 30 mL + R2: 2 x 15 mL |
| 1070179.2RWS | R1: 4 x 34,5 mL + R2: 4 x 10,3 mL |
| 1070100MKWS | R1: 2 x 40 mL + R2: 2 x 10 mL |

### INTENDED USE

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

### SUMMARY [1,2]

Urea is the nitrogen-containing end product of protein catabolism. States associated with high levels of urea in the blood are referred to hyperuremia or azotemia. Parallel determination of urea and creatinine is used in the differentiation between prerenal and postrenal azotemia. Prerenal azotemia caused for example by dehydration, increased protein catabolism, treatment with cortisol or decreased renal perfusion, induces increased urea levels, while creatinine values remain within the reference range. In postrenal azotemia, caused by urinary tract obstruction, the levels of both urea and creatinine rise, but creatinine to a lesser extent. In kidney diseases, urea concentrations are high when there is a reduction in glomerular filtration and when the level of protein ingested is greater than 200 g/day.

### METHOD

Urease – GLDH“: enzymatic UV test

### PRINCIPLE

Urea is hydrolyzed to ammonia by urease. Ammonia reacts with 2-oxoglutarate and NADH in a reaction catalyzed by GLDH promoting the oxidation of NADH to NAD. The consequent reduction in absorbance measured at 340nm is proportional to the concentration of urea.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + 2HCO_3^-$$

$$2-\text{Oxoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L}-\text{Glutamate} + NAD^+ + H_2O$$

GLDH: Glutamate Dehydrogenase

### REAGENTS

**Components and Concentrations**

| | | | |
|---|---|---|---|
| **R1** | TRIS | | **150 mmol/L** |
| | Alpha ketoglutarate | | **<10 mmol/L** |
| | ADP | | **0.75 mmol/L** |
| | Urease | | **<20 KU/L** |
| | Glutamate Dehydrogenase | (GLDH) | **<5 KU/L** |
| **R2** | | | |
| | NADH | | **1.32 mmol/L** |

### 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!

### WARNINGS AND PRECAUTIONS

1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 1 contains material of biological origin. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [3].
4. In case of product malfunction or altered appearance that could affect the performance, contact the manufacturer.
5. Any serious incident related to the product must be reported to the manufacturer.
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.

### 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

Leave the monoreagent for at least 30 min at a temperature of 15 to 25 ºC before use.

| | | | |
|---|---|---|---|
| Stability: | 5 days | at | 15 - 25 ºC |
| | 4 weeks | at | 2 - 8 ºC |

Protect the reagents from light!

### MATERIALS REQUIRED, BUT NOT PROVIDED

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

### SPECIMEN

Serum, plasma (without ammonium heparinate), fresh urine.
Dilute urine 1 + 50 with distilled water and multiply the results by 51.
Use only suitable tubes or collection container for sample collection and preparation.
When using primary tubes, follow the manufacturer's instructions.

Stability [4]

| | | | |
|---|---|---|---|
| In serum or plasma: | 7 days | at | 20 - 25 ºC |
| | 7 days | at | 4 - 8 ºC |
| | 1 year | at | -20 ºC |
| In urine: | 2 days | at | 20 - 25 ºC |
| | 7 days | at | 4 - 8 ºC |
| | 1 month | at | -20 ºC |

Discard contaminated specimens.
Only freeze once!

### ASSAY PROCEDURE

**Applications for automatic systems are available upon request.**

| | |
|---|---|
| Wavelength | 340nm, Hg 334nm, Hg 365nm |
| Optical path | 1 cm |
| Temperature | 25 °C / 30 ºC / 37 ºC |
| Measurement | Against reagent blank<br>Two-point kinetics |

**Starting with Substrate**

| | Blank | Sample or calibrator |
|---|---|---|
| **Sample or calibrator** | - | 10 µL |
| **Reagent 1** | 1000 µL | 1000 µL |
| Mix, incubate for 0 to 5 min, then add: | | |
| **Reagent 2** | 250 µL | 250 µL |
| Mix, incubate for approximately 60 sec. at 25 ºC/30 °C or approximately 30 to 40 sec. at 37°C, then read absorbance A1.<br>Read the absorbance A2 after exactly 60 sec. | | |

ΔA= (A1-A2) Sample

**Starting with Sample**

| | Blank | Sample or calibrator |
|---|---|---|
| **Sample or calibrator** | - | 10 µL |
| **Monoreagent** | 1000 µL | 1000 µL |
| Mix, incubate for approximately 60 sec. at 25 ºC/30 °C or approximately 30 to 40 sec. at 37°C, then read absorbance A1.<br>Read the absorbance A2 after exactly 60 sec. | | |

ΔA= (A1-A2) Sample

**Notes**

1. The method is optimized for 2-point kinetics measurement. It is recommended to perform the test only in automated equipment because it is difficult to incubate **all** samples and blank of the reagent **in exactly the same time interval**. The test design can

be used for adaptation purposes on equipment without a specific adaptation table. Volumes can be proportionately smaller.

2. The statement "approximately 60 sec or approximately 30 – 40 sec" means that the user must necessarily select the pre-incubation time and then this must be **exactly** the same for all samples, standards and for the reagent blank.

## CALCULATION

### With calibrator

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

### Conversion factor

Urea [mg/dL] x 0.1665 = Urea [mmol/L]
Urea [mg/dL] x 0.467 = BUN [mg/dL]
BUN [mgl/dl] x 2.14 = Urea [mg/dL]

(BUN: "Blood Urea Nitrogen")

## 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 urea concentrations within a measurement range of 2 – 300 mg/dL (0.3 – 50 mmol/L). When the values exceed this range, the samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result is multiplied by 3.

### Specificity / Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL, and lipemia up to 2,000 mg/dL of triglycerides. Ammonium ions interfere, so ammonium heparinate should not be used as an anticoagulant for plasma collection! For more information on interfering substances see Young DS [5,6].

### Sensitivity / Limit of Detection

The lowest detection limit is 2 mg/dL.

### Precision (37°C)

| Intra-assay precision n = 10 | | Mean [mg/dL] | SD [mg/dL] | CV [%] |
|---|---|---|---|---|
| Batch N1 | Normal Control | 36.7 | 0.29 | 0.80 |
| Batch P1 | Pathological Control | 152.6 | 0.73 | 0.48 |
| Batch N2 | Normal Control | 35.6 | 0.31 | 0.87 |
| Batch P2 | Pathological Control | 144.0 | 0.78 | 0.54 |

| Inter-assay precision n = 10 | | Mean [mg/dL] | SD [mg/dL] | CV [%] |
|---|---|---|---|---|
| Batch N1 | Normal Control | 36.2 | 0.50 | 1.38 |
| Batch P1 | Pathological Control | 153.2 | 2.68 | 1.75 |
| Batch N2 | Normal Control | 35.4 | 0.38 | 1.07 |
| Batch P2 | Pathological Control | 143.7 | 1.84 | 1.28 |

### Method comparison

Method comparison between Kovalent Ureia UV WS (y) and a commercial test available (x) using 30 samples demonstrated the following results:
$y = 0.9897x + 0.4072$ mg/dL; $R^2 = 0.9971$.

## REFERENCE VALUES

| **In serum/plasma [1]** | **[mg/dL]** | **[mmol/L]** |
|---|---|---|
| **Adults** | | |
| Global | 17 – 43 | 2.8 – 7.2 |
| Women < 50 years | 15 – 40 | 2.6 – 6.7 |
| Women > 50 years | 21 – 43 | 3.5 – 7.2 |
| Men < 50 years | 19 – 44 | 3.2 – 7.3 |
| Men > 50 years | 18 – 55 | 3.0 – 9.2 |
| **Children** | | |
| 1 – 3 years | 11 – 36 | 1.8 – 6.0 |
| 4 – 13 years | 15 – 36 | 2.5 – 6.0 |
| 14 – 19 years | 18 – 45 | 2.9 – 7.5 |

| **BUN in serum/plasma** | **[mg/dL]** | **[mmol/L]** |
|---|---|---|
| **Adults** | | |
| Global | 7.94 – 20.1 | 2.8 – 7.2 |
| Women < 50 years | 7.01 – 18.7 | 2.6 – 6.7 |
| Women > 50 years | 9.81 – 20.1 | 3.5 – 7.2 |
| Men < 50 years | 8.87 – 20.5 | 3.2 – 7.3 |
| Men > 50 years | 8.41 – 25.7 | 3.0 – 9.2 |
| **Children** | | |
| 1 – 3 years | 5.14 – 16.8 | 1.8 – 6.0 |
| 4 – 13 years | 7.01 – 16.8 | 2.5 – 6.0 |
| 14 – 19 years | 8.41 – 21.0 | 2.9 – 7.5 |

**Urea/Creatinine ratio in serum [1]**

25 - 40 [(mmol/L)/(mmol/L)]
20 - 35 [(mg/dL)/(mg/dL)]

**Urea in urine [2]**

26 – 43 g/24h (0.43 – 0.72 mol/24h)

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. 374-7.
2. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1838.3.
3. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.4.
4. Guder WG, da Fonseca-Wollheim F, Heil W, et al. The Quality of Diagnostic Samples. 3rd ed. Darmstadt: GIT Verlag; 2010. p. 62-3; 68-9.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.6.
6. Young DS. Effects on Clinical Laboratory Tests - Drugs Disease, Herbs & Natural Products, https://clinfx.wiley.com/ aaccweb/aacc/, accessed in May 2022. Published by AACC Press and John Wiley and Sons, Inc.

## 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 |
|---|---|
| 80115310189 | R1: 1 x 200 mL + R2: 1 x 50 mL |
| 80115310189 | R1: 1 x 800 mL + R2: 1 x 200 mL |
| 80115310189 | R1: 4 x 28 mL + R2: 4 x 7 mL |
| 80115310189 | R1: 4 x 40 mL + R2: 4 x 10 mL |
| 80115310189 | R1: 5 x 80 mL + R2: 1 x 100 mL |
| 80115310189 | R1: 6 x 39 mL + R2: 6 x 13,7 mL |
| 80115310189 | R1: 8 x 50 mL + R2: 8 x 12,5 mL |
| 80115310189 | R1: 8 x 60 mL + R2: 8 x 15 mL |
| 80115310189 | R1: 10 x 20 mL + R2: 2 x 25 mL |
| 80115310189 | R1: 10 x 20 mL + R2: 2 x 30 mL |
| 80115310189 | R1: 3 x 40 mL + R2: 3 x 10 mL |
| 80115310189 | R1: 1 x 40 mL + R2: 1 x 10 mL |

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

**Expiration date and Lot no.: See label**