



# Urea CT\* FS\*\*

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

## Order Information

Cat. No.	Kit size
1 3115 99 10 021	R1 2 x 25 mL + R2 2 x 25 mL + R3 1 x 0.5 mL + 1 x 3 mL Standard
1 3115 99 10 026	R1 3 x 100 mL + R2 3 x 100 mL + R3 2 x 1.5 mL
1 3115 99 90 305	R1 6 x 25 mL + R2 6 x 25 mL + R3 1 x 1.5 mL
1 3100 99 10 030	6 x 3 mL Standard

## Summary [1,2]

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyperuremia or azotemia. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia. Pre-renal azotemia, caused by e.g. dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion, leads to increased urea levels, while creatinine values remain within the reference range. In post-renal azotemias, caused by the obstruction of the urinary tract, both urea and creatinine levels rise, but creatinine in a smaller extent. In renal diseases urea concentrations are elevated when the glomerular filtration rate is markedly reduced and when the protein intake is higher than 200 g/day.

## Method

Colorimetric test

## Principle

Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. Ammonium ions react with hypochlorite and salicylate to give a green dye. The increase in absorbance at 578 nm is proportional to the urea concentration in the sample.

## Reagents

### Components and Concentrations

<b>R1:</b>	Phosphate buffer	120 mmol/L
	Sodium salicylate	60 mmol/L
	Sodium nitroprusside	40 mmol/L
	EDTA	1.3 mmol/L
<b>R2:</b>	Phosphate buffer	< 50 mmol/L
	Sodium hydroxide	150 mmol/L
	Sodium hypochlorite	10 mmol/L
<b>R3:</b>	Urease	≥ 0.5 kU/mL
<b>Standard:</b>		50 mg/dL (8.33 mmol/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 and contamination is avoided.

The reagents must be protected from light!

Do not freeze the reagents!

The standard is stable up to the end of the indicated month of expiry, if stored at 2 – 25 °C.

## Warnings and Precautions

1. Standard and R3 contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Reagent 2 is irritating. R36/38: Irritating to eyes and skin. S2: Keep out of the reach of children. S24/25: Avoid contact with skin and eyes. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.
3. Reagent 3 is harmful. R42: May cause sensitisation by inhalation. S2: Keep out of the reach of children. S23: Do not breathe gas/fumes/vapour/spray (appropriate wording to be specified by the manufacturer). S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). S63: In case of accident by inhalation: remove casualty to fresh air and keep at rest.
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

## Waste Management

Please refer to local legal requirements.

## Reagent Preparation

Mix R1 + R3 in the ratio 100 + 1

e.g. 20 mL R1 + 0.2 mL R3 = R1A

Stability of R1A:

2 weeks at 2 – 8 °C

2 days at 15 – 25 °C

R1A and R2 must be protected from light!

Reagent 2 and standard are ready for use.

## Materials required but not provided

NaCl solution 9 g/L

General laboratory equipment

## Specimen

Serum, EDTA plasma and heparin plasma (no ammonium heparin!), urine

Dilute urine 1 + 100 with dist. water and multiply results by 101.

Stability in serum or plasma [5]:

7 days at 20 – 25 °C

7 days at 4 – 8 °C

1 year at -20 °C

Stability in urine [5]:

2 days at 20 – 25 °C

7 days at 4 – 8 °C

1 month at -20 °C

Discard contaminated specimens!

## Assay Procedure

**Application sheets for automated systems are available on request.**

Wavelength	578 nm, 560 – 600 nm
Optical path	1 cm
Temperature	20 – 25 °C, 37 °C
Measurement	Against reagent blank

Only one reagent blank per series is required.

	Blank	Sample or standard
<b>Sample or standard</b>	-	10 µL
<b>Reagent 1A</b>	1000 µL	1000 µL
Mix, incubate 10 min. at 20 – 25 °C or 5 min. at 37 °C, then add:		
<b>Reagent 2</b>	1000 µL	1000 µL
Mix, incubate 10 min. at 20 – 25 °C or 5 min. at 37 °C. Read the absorbance within 30 min. against the reagent blank.		

## Calculation

With standard or calibrator

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

## Conversion factor

$$\text{Urea [mg/dL]} \times 0.1665 = \text{Urea [mmol/L]}$$

$$\text{Urea [mg/dL]} \times 0.467 = \text{BUN [mg/dL]}$$

$$\text{BUN [mg/dL]} \times 2.14 = \text{Urea [mg/dL]}$$

(BUN: Blood urea nitrogen)

## Calibrators and Controls

For the calibration of automated photometric systems the DiaSys TruCal U calibrator is recommended. For internal quality control commercially available controls should be assayed with each batch of samples.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 X 3 mL
	5 9100 99 10 064	6 x 3 mL

## Performance Characteristics

### Measuring range

The test has been developed to determine urea concentrations within a measuring range from 1 – 400 mg/dL (0.17 – 67 mmol/L) in serum/plasma or 40 g/dL (6.7 mol/L) in urine. When values exceed this range the samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result 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 200 mg/dL and lipemia up to 800 mg/dL triglycerides. Ammonium ions interfere, therefore do not use ammonium heparin as anticoagulant for collection of plasma.

### Sensitivity/Limit of Detection

The lower limit of detection is 1 mg/dL.

## Precision (at 20 - 25 °C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	27.3	0.38	1.38
Sample 2	39.0	0.54	1.39
Sample 3	149	2.50	1.68

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	21.1	0.74	3.51
Sample 2	43.8	1.01	2.31
Sample 3	145	3.50	2.41

## Method Comparison

A comparison of DiaSys Urea CT FS (y) with a kinetic test (x) using 66 samples gave following results:  
 $y = 1.03 x + 0.32 \text{ mg/dL}; r = 0.996.$

## Reference Range

### In Serum/Plasma [1]

	[mg/dL]	[mmol/L]
<b>Adults</b>		
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
<b>Children</b>		
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

### Urea/Creatinine ratio [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. 1<sup>st</sup> ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 374-7.
2. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3<sup>rd</sup> ed. Philadelphia: W.B Saunders Company; 1999. p. 1838.
3. Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. Clin Path 1960;13:156-9.
4. Patton CJ, Crouch SR. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of urea. Anal Chem 1977;49:464-9.
5. Guder WG, Zatwa B et al. The quality of Diagnostic Samples. 1<sup>st</sup> ed. Darmstadt: Git Verlag, 2001; p. 48-9, 52-3.

## Manufacturer

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