



Urea 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 3101 99 10 021	R1 4 x +	20 mL	+	R2 1 x 3 mL Standard	20 mL
1 3101 99 10 026	R1 5 x	80 mL	+	R2 1 x	100 mL
1 3101 99 10 023	R1 1 x	800 mL	+	R2 1 x	200 mL
1 3101 99 10 704	R1 8 x	50 mL	+	R2 8 x	12.5 mL
1 3101 99 10 917	R1 8 x	60 mL	+	R2 8 x	15 mL
1 3101 99 10 191	R1 4 x	36 mL	+	R2 4 x	9 mL
1 3101 99 90 314	R1 10 x	20 mL	+	R2 2 x	30 mL
1 3101 99 10 950	3700 Tests on ADVIA 1650/1800				
1 3100 99 10 030	6 x	3 mL		Standard	

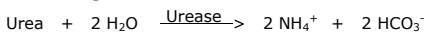
Summary [1,2]

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

"Urease - GLDH": enzymatic UV test

Principle



GLDH: Glutamate dehydrogenase

Reagents

Components and Concentrations

R1:	TRIS	pH 7.8	150 mmol/L
	2-Oxoglutarate		9 mmol/L
	ADP		0.75 mmol/L
	Urease		≥ 7 kU/L
	GLDH (Glutamate dehydrogenase)		≥ 1 kU/L
R2:	NADH		1.3 mmol/L
Standard:		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. 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

- The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
- 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

Substrate Start

The standard and the reagents are ready to use.

Sample Start

Mix 4 parts of R1 with 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono-reagent
Leave the mono-reagent for at least 30 min. at 15 – 25 °C before use.

Stability: 4 weeks at 2 – 8 °C
5 days at 15 – 25 °C

Protect the monoreagent from light!

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, plasma (no ammonium heparin!), fresh urine
Dilute urine 1 + 100 with dist. water and multiply results by 101.

Stability [4]

in serum or plasma:

7 days at 20 – 25 °C
7 days at 4 – 8 °C
1 year at -20 °C

in urine:

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	340 nm, Hg 334 nm, Hg 365 nm
Optical path	1 cm
Temperature	25 °C/30 °C/37 °C
Measurement	Against reagent blank 2-point kinetic

Substrate start

	Blank	Sample or standard
Sample or standard	-	10 µL
Reagent 1	1000 µL	1000 µL
Mix, incubate 0 – 5 min., then add:		
Reagent 2	250 µL	250 µL
Mix, incubate for approx. 60 sec. at 25 °C/30 °C or approx. 30 - 40 sec at 37 °C, then read absorbance A1.		
After exactly further 60 sec. read absorbance A2.		

ΔA = (A1 – A2) sample or standard

Sample start

	Blank	Sample or standard
Sample or standard	-	10 µL
Mono-reagent	1000 µL	1000 µL
Mix, incubate for approx. 60 sec. at 25 °C/30 °C or approx. 30 - 40 sec at 37 °C, then read absorbance A1.		
After exactly further 60 sec. read absorbance A2.		

ΔA = (A1 – A2) sample or standard

Notes

1. The method is optimized for 2-point kinetic measurement. It is recommended to perform the method only on mechanized equipment because it is difficult to incubate **all** samples and the reagent blank **exactly** for the same time intervals. The assay scheme may be used for adaptation purposes for instruments with no specific adaptation sheet. The volumes may be proportionally smaller.
2. The statement "approx. 60 sec. at 25 °C/30 °C or approx. 30 - 40 sec at 37 °C" means that the user must select the necessary preincubation time and then this must be exactly the same for all the tests.

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 DiaSys TruLab N, P and TruLab Urine 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
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL
TruLab Urine Level 1	5 9170 99 10 062	20 x 5 mL
	5 9170 99 10 061	6 x 5 mL
TruLab Urine Level 2	5 9180 99 10 062	20 x 5 mL
	5 9180 99 10 061	6 x 5 mL

Performance Characteristics

Measuring range

The test has been developed to determine urea concentrations within a measuring range from 2 - 300 mg/dL (0.3 - 50 mmol/L) in serum/plasma or 30 g/dL (5 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 500 mg/dL and lipemia up to 2,000 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 2 mg/dL.

Precision (at 37°C)

Intra-assay precision n = 20	Mean [mg/dL]	SD [.mg/dL]	CV [%]
Sample 1	29.8	1.61	5.41
Sample 2	52.7	1.65	3.13
Sample 3	117	1.48	1.27

Inter-assay precision n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	31.4	1.82	5.79
Sample 2	52.7	2.04	3.87
Sample 3	117	3.35	2.86

Method Comparison

A comparison of DiaSys Urea FS (y) with a commercially available test (x) using 68 samples gave following results: $y = 0.99x + 1.06 \text{ mg/dL}$; $r = 0.999$.

Reference Range

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

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. 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. Talke H, Schubert GE. Enzymatische Harnstoffbestimmung in Blut und Serum im optischen Test nach Warburg (Enzymatic determination of urea in blood and serum with the optical test according to Warburg). Klin Wschr 1965; 43: 174-5.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p. 48-9, 52-3.

Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany