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Urea UV Auto

Urease / GLDH

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

REF	Kit Size	Configuration
D03121B	1 x 1.25 L	1 x 1 L R1 + 1 x 0.25 L R2
D95704	5 x 100 mL	4 x 100 mL R1 + 1 x 100 mL R2
D98707	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
D00715	5 x 25 mL	4 x 25 mL R1 + 1 x 25 mL R2
D00716	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
D82911	10 x 50 mL	10 x 40 mL R1 + 4 x 25 mL R2
D0439917	5 x 62.5 mL	4 x 62.5 mL R1 + 1 x 62.5 mL R2
DA0845	5 x 50 mL	5 x 40 mL R1 + 5 x 10 mL R2
DT1045	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2
DK0742	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DE1845	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2
DB20333	4 x 62.5 mL	4 x 50 mL R1 + 4 x 12.5 mL R2

Additionally available:

D95706	1 x 3 mL	Urea Standard	
D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P
D08581	12 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08581SV	1 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08582	12 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2
D08582SV	1 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	UV, 2 Point Kinetic (fixed time), decreasing reaction, GLDH
Shelf life	24 months
Storage	2 – 8°C
Wavelength	340 nm, Hg 334 nm, Hg 365 nm
Temperature	25 °C, 30 °C or 37 °C
Sample	Serum, plasma, urine

INTENDED USE

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

DIAGNOSTIC SIGNIFICANCE [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 uea 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, for example 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.

TEST PRINCIPLE

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

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

Decrease in absorbance, resulting from the GLDH-reaction, is proportional to the concentration of Urea in the sample.

NAD = Nicotinamide Adenine Dinucleotide
NADH = reduced NAD
GLDH = Glutamate Dehydrogenase
ADP = Adenosine diphosphate

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Reagent 1:		
Tris buffer, pH 7.8	150	mmol/L
2-Oxoglutarate	9	mmol/L
ADP	0.75	mmol/L
Urease	≥ 7	kU/L
GLDH (Glutamate dehydrogenase, bovine)	≥ 1	kU/L
Reagent 2		
NADH	1.3	mmol/L

MATERIAL REQUIRED BUT NOT PROVIDED

- NaCl solution (9 g/L).
- Clinical chemistry analyser.

REAGENT PREPARATION

Substrate Start:
Reagents are ready to use.

Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2 (= Working reagent). Leave the working reagent for at least 30 min. at 15 – 25 °C before use.

STORAGE AND STABILITY

Conditions: Protect from light!
Close immediately after use
Do not freeze the reagents!
Avoid contamination

Substrate Start:
Storage: at 2 – 8 °C
Stability: up to the expiration date indicated on labels
Sample Start (Working Reagent):
Stability: at 15 – 25 °C 5 days
at 2 – 8 °C 4 weeks
Protect the working reagent from light!

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 animal material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
5. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Sample preparation (Urine): Dilute urine 1 + 50 with dist. water and multiply the results by 51. Use fresh urine!
Diacon Urine controls must be prediluted the same way as patient samples.

Do not use ammonium heparin plasma!

Stability [4]:

in serum / plasma:	at 20 – 25 °C	7 days
	at 4 – 8 °C	7 days
	at -20°C	1 year
in Urine	at 20 – 25 °C	2 days
	at 4 – 8 °C	7 days
	at -20°C	1 month

Freeze only once! Discard contaminated specimens.

STANDARD

(not included in the kits; has to be ordered separately)
Concentration 50 mg/dL (8.33 mmol/L)
Storage: 2 – 8 °C
Stability: up to the indicated expiration date
Close immediately after use! Avoid contamination! Protect from light!

TEST PROCEDURE

Bring reagents and samples to room temperature.

Reagent start

Pipette into test tubes	Blank	Std./Cal.	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard/Calibrator	-	10 µl	-
Mix. Incubate for 0 – 5 minutes, then add:			
Reagent 2	250 µl	250 µl	250 µl
Mix, incubate for approx. 60 sec. at 25/30 °C or approx. 30 – 40 sec. at 37 °C and measure absorbance A1 against reagent blank. Incubate for exactly 60 sec. and measure absorbance A2 against reagent blank. Calculate ΔA = (A1 – A2) Sample or Std./Cal.			

Sample start

Pipette into test tubes	Blank	Std./Cal.	Sample
Working Reagent	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Stdandard/Calibrator	-	10 µl	-
Mix, incubate for approx. 60 sec. at 25/30 °C or approx. 30 – 40 sec. at 37 °C and measure absorbance A1 against reagent blank. Incubate for exactly 60 sec. and measure absorbance A2 against reagent blank. Calculate ΔA = (A1 – A2) Sample or Std./Cal.			

Note:
1.The method is optimized for 2-point kinetic measurement. It is mandatory to incubate all samples and the reagent blank **strictly** for the same time intervals. This method is therefore recommended only for automated test procedure on automatic analysers.

2. The statement "approx. 60 sec. or approx. 30 - 40 sec." means that the time period chosen does not need to be exactly 60 or 30 – 40 sec, respectively. A time period once chosen (e.g. 55 sec.) has to be respected **exactly** for all samples, Std./Cal. and the reagent blank.

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation
With standard or calibrator:
Serum/Plasma:

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

Urine:

$$\text{Urea [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]} \times 51$$

Unit Conversion
Urea [mg/dL] x 0.1665 = Urea [mmol/L]
Urea [mg/dL] x 0.467 = BUN [mg/dL]
BUN [mg/dL] x 2.14 = Urea [mg/dL]
(BUN: Blood Urea Nitrogen)

QUALITY CONTROL AND CALIBRATION

All controls with urea values determined by this method can be used.
We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range) as well as the Dialab urine controls **Diacon Urine Level 1** (control urine normal) and **Level 2** (control urine abnormal).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The assay requires the use of a uric acid standard or calibrator. We recommend the Dialab **Urea Standard** and the Dialab multi calibration serum **Diacal Auto**.

PERFORMANCE CHARACTERISTICS

LINEARITY, 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 respectively up to 30 g/dL (5 mol/L) in urine. If values exceed this range the samples should be diluted 1 + 2 with NaCl solution (9 g/L) and the result multiplied by 3.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 2 mg/dL.

PRECISION (at 37°C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	21.3	0.50	2.33
Sample 2	35.3	0.82	2.33
Sample 3	141	1.52	1.08

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	20.3	0.58	2.88
Sample 2	48.3	1.12	2.32
Sample 3	152	1.38	0.91

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	2000 mg/dL

Ammonium ions interfere, therefore do not use ammonium herparin as anticoagulant for collection of plasma!

For further information on interfering substances refer to Young DS [5].

METHOD COMPARISON

A comparison between Dialab Urea (y) and a commercially available test (x) using 68 samples gave following results: y = 0.99 x + 1.06 mg/dL; r = 0.999.

TRACEABILITY

The assigned values of the calibrator have been made traceable to NIST SRM®-909 Level 1.

EXPECTED VALUES*

In serum / plasma [1]:

Adults:	[mg/dL]	[mmol/L]
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:

Adults:	[mg/dL]	[mmol/L]
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.

LIMITATIONS

- Eventual Urea UV Auto, Urease/GLDH carry-over to reagents Phosphorus Inorganic (Molybdate), Bilirubin Auto Total (DCA) and Protein Total in Urine/CSF (Pyrogallol red). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p. 374-7.
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4. Guder WG, Zawta Be et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001. p. 48-9.
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.
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CE IVD 2°C 8°C