UREA

Cat. No.	Pack Name	Packaging (Content)
BLT00060	UREA 1000	R1: 4 x 200 ml, R2: 1 x 200 ml
BLT00061	UREA 250	R1: 4 x 50 ml, R2: 1 x 50 ml, R3 standard 1 x 5 ml

EN

CE IVD

INTENDED USE
Diagnostic reagent for quantitative *in vitro* determination of Urea in human serum, plasma and urine.

CLINICAL SIGNIFICANCE
Urea is the major end product of protein nitrogen metabolism in humans. It constitutes the largest fraction of the nonprotein nitrogen component of the blood. Urea is produced in the liver and excreted through the kidneys in the urine. Consequently, the circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur with dietary changes, diseases which impair kidney function, liver diseases, congestive heart failure, diabetes and infections.

PRINCIPLE
The enzyme methodology employed in this reagent is based on the reaction first described by Talke and Schubert. To shorten and simplify the assay, the calculations are based on the discovery of Tiffany et al. that urea concentration is proportional to absorbance change over a fixed time interval.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-KG} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + \text{NAD}$$

1. Urea is hydrolysed in the presence of water and Urease to produce ammonia and carbon dioxide.
2. In the presence of Glutamate Dehydrogenase (GLDH) and reduced Nicotinamide Adenine Dinucleotide (NADH), ammonia combines with α-ketoglutarte (α-KG) to produce L-Glutamate.
3. The reaction is monitored by measuring the rate of decrease in absorbance at 340 nm as NADH is converted to NAD.

REAGENT COMPOSITION
R1

Tris Buffer	100 mmol/l
α-Ketoglutarate	5.49 mmol/l
Urease (Jack Bean)	≥ 10 kU/l
GLDH (Microorganism)	≥ 3.8 kU/l

R2

NADH	1.66 mmol/l

Also contains Non-reactive fillers and stabilizers.

R3 standard See bottle label

REAGENT PREPARATION
Reagents are liquid, ready to use.

STABILITY AND STORAGE
The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2–8°C.

Two reagents method – substrate start
Reagents are ready to use.
After opening, reagents are stable until expiry date at 2–8°C if stored at appropriate conditions, closed carefully and without any contamination.

Monoreagent method – sample start
Mix 4 portion of reagent R1 with 1 portion of reagent R2.

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

SPECIMEN COLLECTION AND HANDLING
Use serum, EDTA plasma and heparin (no ammonium heparin!) plasma, urine.
It is recommended to follow NCCLS procedures (or similar standardized conditions).
Dilute urine 1 + 100 with dist. water and multiply results by 101.

Stability

in serum/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
	2 days	at	4–8°C
	1 month	at	-20°C

Discard contaminated specimens.

CALIBRATION
Calibration with the standard included in the kit or calibrator XL MULTICAL, Cat. No. XSYS0034 is recommended.

QUALITY CONTROL
For quality control ERBA NORM, Cat. No. BLT00080 and ERBA PATH, Cat. No. BLT00081 are recommended.

UNIT CONVERSION
mg/dl x 0.1665 = mmol/l
Urea (mg/dl) x 0.467 = BUN (mg/dl)
BUN (mg/dl) x 2.14 = Urea (mg/dl)

EXPECTED VALUES [1,2]
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

Urea / Creatinine ratio [1]
20 – 35 [(mg/dl)/(mg/dl)]

Urea in Urine [2]
26 – 43 g/24 h (0.43 – 0.72 mol/24 h)

It is recommended that each laboratory verify this range or derives reference interval for the population it serves.

PERFORMANCE DATA
Data contained within this section is representative of performance on ERBA XL systems. Data obtained in your laboratory may differ from these values.

Limit of quantification: 11.5 mg/dl

Linearity: 300 mg/dl of Urea or
140 mg/dl of Urea Nitrogen

Measuring range: 11.5 - 300 mg/dl

PRECISION

Intra-assay precision Within run (n=20)	**Mean (mg/dl)**	**SD (mg/dl)**	**CV (%)**
Sample 1	28.08	0.287	1.02
Sample 2	27.49	0.240	0.94

Inter-assay precision Run to run (n=20)	**Mean (mg/dl)**	**SD (mg/dl)**	**CV (%)**
Sample 1	45.09	0.719	1.61
Sample 2	203.59	2.395	1.58

COMPARISON
A comparison between XL-Systems Urea (y) and a commercially available test (x) using 40 samples gave following results:

y = 1.034 x - 0.295 mg/dl
r = 0.994

INTERFERENCES
Following substances do not interfere:
haemoglobin up to 7.5 g/l, bilirubin up to 30 mg/dl, triglycerides up to 2000 mg/dl

WARNING AND PRECAUTIONS
For *in vitro* diagnostic use. To be handled by entitled and professionally educated person.
Reagents of the kit are not classified like dangerous but contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

WASTE MANAGEMENT
Please refer to local legal requirements.

ASSAY PROCEDURE
Wavelength **340 nm**
Cuvette **1 cm**
Two reagents method – substrate start

	Reagent blank	Standard	Sample
Reagent 1	1.000 ml	1.000 ml	1.000 ml
Sample	-	-	0.010 ml
Standard	-	0.010 ml	-
Distilled water	0.010 ml	-	-
Mix and after 1 min. incubation (at 37 °C) add:			
Reagent 2	0.250 ml	0.250 ml	0.250 ml

Mix and measure the initial absorbance after 30 sec (A_1), start timer simultaneously and read again exactly after 1 min (A_2). Measure against reagent blank.
Calculate absorbance change $\Delta A_{sam} = (A_2 - A_1)$/min.

Erba Lachema s.r.o., Karásek 1d, 621 00 Brno, CZ
e-mail: diagnostics@erbalachema.com, www.erbamannheim.com

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Monoreagent method – sample start

	Reagent blank	Standard (Cal.)	Sample
Working reagent	1.000 ml	1.000 ml	1.000 ml
Sample	-	-	0.010 ml
Standard (Cal.)	-	0.010 ml	-
Distilled water	0.010 ml	-	-

Mix and measure the initial absorbance after 30 sec (A_1), start timer simultaneously and read again exactly after 1 min (A_2). Measure against reagent blank.

Calculate absorbance change $\Delta A_{sam} = (A_2 - A_1)/min$.

CALCULATION

$$\text{Urea (mg/dl)} = \frac{\Delta A_{sam} - \Delta A_{bl}}{\Delta A_{cal} - \Delta A_{bl}} \times C_{cal}$$

C_{cal} = calibrator (standard) concentration

Applications for automatic analysers are available on request.

ASSAY PARAMETERS FOR PHOTOMETERS

Mode	Fixed time
Wavelength 1 (nm)	340
Sample Volume (µl)	5/10
Working Reagent Volume (µl)	500/1000
Lag time (sec.)	20
Kinetic interval (sec.)	60
No. of readings	1
Reaction temperature (°C)	37
Reaction direction	Decreasing
Normal Low (mg/dl)	17
Normal High (mg/dl)	43
Linearity Low (mg/dl)	11.5
Linearity High (mg/dl)	300
Blank with	Reagent
Absorbance limit (max.)	1.1
Concentration of Standard	See bottle label
Units	mg/dl

REFERENCES

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SYMBOLS USED ON LABELS

Catalogue Number


Manufacturer


See Instruction for Use

Lot Number


CE Mark - Device comply with the Directive 98/79/EC


Storage Temperature


Expiry Date

In Vitro Diagnostics

Content

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

Erba Lachema s.r.o., Karásek 1d, 621 00 Brno, CZ
e-mail: diagnostics@erbalachema.com, www.erbamannheim.com