BIOLABO
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UREA U.V. High Linearity Kinetic Method

Reagent for quantitative determination of urea (UREA) in human serum and plasma or urines.

REF LP99532	**R1** 4 x 30 mL	**R2** 1 x 30 mL	**R3** 1 x 10 mL
REF LP99632	**R1** 4 x 100 mL	**R2** 1 x 100 mL	**R3** 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
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Latest revision: www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (automated method).
It allows the quantitative determination of urea (UREA) in human serum and plasma or urines.

I GENERALITIES (1) (6)

More than 90% of urea is excreted through the kidneys in urines. Measurement of the plasma or serum urea concentration is widely regarded as a test of renal function. However, a number of non-renal factors also influence the circulating urea concentration: Urea increased level occurs when proteins catabolism is accelerated, burns, stress, myocardial infarction... Urea is decreased in acute liver destruction and is accompanied with increased ammonium level. Urea level is generally studied in conjunction with creatinine level (urea/creatinine ratio) to refine the diagnosis of post-renal or pre-renal azotemia.

PRINCIPLE (4) (5)

Enzymatic method based on Talke and Schubert reaction, simplified by Tiffany and al. who demonstrated that urea concentration is proportional to absorbance change at 340 nm over a fixed time interval. Reaction scheme is as follows:

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

$$NH_3 + \text{oxoglutarate} + \text{NADH} + H^+ \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+ + H_2O$$

REAGENTS

R1 UREA BUF ENZ — Buffer Enzymes

Tris pH 7.9 ± 0.1 at 30°C	100	mmol/L
Urease	≥ 17000	IU/L
GLDH	≥ 700	IU/L
Oxoglutarate	6.5	mmol/L
Preservative		

R2 UREA COENZ — Coenzyme

NADH	≥ 1.5	mmol/L
Preservative		

R3 UREA — Standard

Urea	40 mg/dL	(6.66 mmol/L)

According to 1272/2008/EC Regulation, these reagents are not classified as dangerous

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

REF LP99532: Mix 4 volumes of R1 (Buffer-Enzymes) with 1 volume of R2 (Coenzyme).

REF LP99632: Add exactly 25 mL of the contents of vial R2 into vial R1. Well recap and mix gently.

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert:

Unopened:
- Until expiry date stated on the label of the kit.

Once opened:
- Working reagent (R1+R2) is stable for 1 month when free from contamination.
- Discard any cloudy reagent or if reagent blank is < 1.100 at 340 nm.

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum or heparinised plasma. Avoid fluoride or ammonium as anticoagulants which interfere with the assay.

- Stable for 24 h at room temperature
- several days at 2-8°C
- at least 2-3 months frozen

24h Urines:

- Stable for 4 days at 2-8°C
- Add antibacterial agent as Thymol to improve the stability
- Dilute (1+19) with demineralised water before assay

LIMITS (3)

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Thermostated Spectrophotometer or Biochemistry Analyzer

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

QUALITY CONTROL

- REF 95010 EXATROL-N Level I
- REF 95011 EXATROL-P Level II
- REF 95012 Urinary Controls
- External quality control program

It is recommended to control in the following cases:

- At least once a run
- At least once within 24 hours
- When changing vial of reagent
- After maintenance operations on the instrument

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

Serum or plasma	(mg/dL)	[mmol/L]
In cord	45-86	[7.5-14.3]
Premature	6-54	[1.1-8.9]
< 1 year	9-41	[1.4-6.8]
Children	11-39	[1.8-6.4]
18-60 years	13-43	[2.1-7.1]
60-90 years	17-49	[2.9-8.2]
> 90 years	21-66	[3.6-11.1]

Urines	26-43 g/24 h	[0.43-0.71 mol/24 h]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

On Kenza 240TX, at 37°C, 340nm:

Linearity Range: between 11 and 250 mg/dL

Detection limit: approx. 1.7 mg/dL

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	14.0	40.8	123.9
S.D. mg/dL	0.5	0.6	2.1
C.V. %	3.5	1.5	1.7

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	15.1	43.4	132.2
S.D. mg/dL	0.5	1.1	2.8
C.V. %	3.3	2.6	2.1

Comparison studies with commercially available reagent:
Realised on human specimens (n=100) between 12 and 300 mg/dL

y = 1.0249 x – 1.0527 r = 0.9990

Analytical Sensitivity: approx. 0.0012 abs/min for 1 mg/dL

Interferences:

Total bilirubin	No interference up to 502 µmol/L
Direct bilirubin	No interference up to 403 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 1064 mg/dL
Turbidity	Positive interference from 0.143 OD
Haemoglobin	No interference up to 379 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 7 days

Calibration Stability: 7 days

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

Performances and stability data on Kenza 450TX/ISE and Kenza One are available on request.

CALIBRATION (7)

- REF 95015 Multicalibrator traceable to SRM 909c

Or

- Standard (vial R3): manual procedure and urines

The calibration frequency depends on proper instrument functions and on the preservation of reagent
Detailed Kenza 240TX procedure is available on request

PROCEDURE

Manual method:

Let stand reagents and specimens at room temperature.

Pipette into thermostated cuvette (37°C):	Standard	Assay
Reagent	1 mL	1 mL
Standard	10 µL	
Specimen (1)		10 µL
Mix. Start a timer. After 30 seconds, record absorbance A1 at 340 nm and then absorbance A2 after 90 seconds.		

1. Serum, plasma or urines diluted (1+19) in demineralised water.
2. Performances with manual procedure should be validated by user.
3. Kenza applications and other proposal are available on request.

CALCULATION

Manual Procedure

- Serum and plasma:

$$\text{Result} = \frac{\text{Abs (A1 – A2) Assay}}{\text{Abs (A1 – A2) Standard}} \times \text{Standard concentration}$$

To calculate blood urea nitrogen (BUN): multiply the value of urea (mg/dL) by 0.467.

- Urines diluted (1+19):

Multiply the result by 20 (dilution factor).

Automatic Biochemistry analyzer:

The analyzer provides directly final result.

For more details about calibration and calculation of results, refer to User's manual and specific application.

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1239-1241.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 1096-1099.*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1990) p. 3-599 to 3-609*
(4) *Talke H., Schubert G. E., Klin. Wochschr., 19, (1965), 43, p.174*
(5) *Tiffany T. O., and al., Clin. Chem., 18, (1972) p.829-840*
(6) *Bernard S. Bioch. clin. Diagnostics médicaux chirurgicaux 2ème éd. p.143-144. Ed. Maloine PARIS (1989).*
(7) *SRM: Standard Reference Material* ®