BIOLABO
www.biolabo.fr

MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

UREA U.V.

High Linearity Kinetic Method

Reagent for quantitative determination of urea in human serum, plasma or urines.

REF 99032	**R1** 8 x 30 mL	**R2** 8 x 30 mL	**R3** 1 x 10 mL
REF 99132	**R1** 10 x 100 mL	**R2** 10 x 100 mL	**R3** 1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (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:

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

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

REAGENTS COMPOSITION

Vial R1 **TRIS BUFFER**

Tris pH 7.9 ± 0.1 at 30°C	80	mmol/L
Oxoglutarate	5	mmol/L
Preservative		

Vial R2 **ENZYMES COENZYME**

NADH	≥ 0.2	mmol/L
Urease	20000	IU/L
GLDH	≥ 600	IU/L

Vial R3 **STANDARD**

Urea 40 mg/dL (6.66 mmol/L)

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Vial R2: Use a non-sharp instrument to remove aluminium cap.
Add promptly the contents of vial R2 (Enzymes-Coenzyme) into vial R1 (Buffer).

Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).

STABILITY AND STORAGE

Store away from light, well cap in the original vial at 2-8°C.

- Standard (vial R3): Transfer the requested quantity, recap and store at 2-8°C.
- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 1 month when free from contamination.
- Discard any reagent if cloudy or if absorbance of working reagent at 340 nm is < 1.100.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

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

Urea is stable in serum or plasma for:

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

24h Urines: diluted (1+19) with demineralised water before assay.

Urea is stable in urines for:

- 4 days at 2-8°C.

Add antibacterial agent as Thymol to improve the stability.

INTERFERENCES (3)

Bilirubin: No interference up to 30 mg/dL of bilirubin.

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

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control seral

CALIBRATION (7)

- Standard enclosed in the kit (vial R3) or BIOLABO Multicalibrator REF 95015 traceable to SRM 909b.
- Or any calibrator traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of reagent.

It is recommended to calibrate in the following cases :

1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. If control values are out of ranges, even after using a new vial of fresh serum.

QUALITY CONTROL

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- Other assayed control sera referring to the same method.
- 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. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (4)

UREA

In serum and 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]
In urines	26-43 g/24 h	[0.43-0.71 mol/24 h]

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

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

PERFORMANCES CHARACTERISTICS

With Procedure n°2 (at 37°C):

Within run N = 20	*Medium level*	*High level*
Mean mg/dL	47	126
S.D. mg/dL	1.22	1.81
C.V. %	2.59	1.44

Between run N = 30	*Medium level*	*High level*
Mean mg/dL	41.7	137
S.D. mg/dL	2.55	4.15
C.V. %	6.1	3.03

Detection limit: approximately 11 mg/dL.

Sensitivity for 100 mg/dL: 0.062 to 0.125 Abs/min at 340 nm.

Comparison study with commercially available reagent:

y = 0.94 x + 0.02 r = 0.9964

LINEARITY

Procedure n°1: linear up to 600 mg/dL (100 mmol/L).
Procedure n°2: linear up to 300 mg/dL (50 mmol/L).

Above, dilute the specimen with saline solution 9 g/L and reassay taking into account the dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Procedure n°1

Pipette into cuvettes thermostated at 30°C (37°C):	Standard	Assay
Reagent	1 mL	1 mL
Standard	5 µL	
Specimen (Note 1)		5 µL

Mix. Start a timer.
After 30 seconds, record initial absorbance A1 at 340 nm against distilled water.
Record the absorbance A2 after 90 seconds.

Procedure n°2

Pipette into cuvettes thermostated at 30°C (37°C):	Standard	Assay
Reagent	1 mL	1 mL
Standard	10 µL	
Specimen (Note 1)		10 µL

Mix. Start a timer.
After 30 seconds, record initial absorbance A1 at 340 nm against distilled water.
Record the absorbance A2 after 90 seconds.

Notes:

1. Serum, plasma or urines diluted (1+19) in demineralised water.
2. Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

Serum and plasma:

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

Urines diluted (1+19): Multiply the result by 20 (dilution factor).

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 Referance Material ®*