Urea (Urea)
(Urease-GLDH/Kinetic Method)

- **Kit Specifications:**

	Reagent/Quantity	Storage
Cat. No.: **UR0011**	R.1: 4 x 50 ml R.2: 1 x 50 total 250 ml	2-8°C
Cat. No.: **UR0017**	R.1: 4 x 50 ml R.2: 1 x 50 total 250 ml	2-8°C

- **Intended Use:**

In Vitro Diagnostic reagent pack for the quantitative determination of Urea in human serum/plasma and urine on automated and semi-automated photometric systems.

- **Summary and Explanation:**

Urea is prepared in liver with CO2 is a final product of protein metabolism. The amount of urea secretion is depending on many things such as high protein diet, fever, diabetes and hyper activity of adrenal glands have direct relation of this process. The biosynthesis of urea almost done by the liver enzymes in the urea cycle. Also in protein and amino acid nitrogen catabolism convert to urea. The 90 percent of this product remove by kidney and the rest secreted by skin and digestion apparatus. Urea is not going re uptake and re secreted but all be filtered by glomeruli. In the BUN assay the nitrogen of urea will be measured. This is one of the index of glomeruli function, production and secretion of urea. The rapid catabolism of protein and dysfunction of kidney may increase the amount of BUN. Therefore, the rapid of urea production cause tissue necrosis, protein catabolism and increase secretion nitrogen of urea from kidneys. The BUN assay is less sensitive compare to the creatinine clearance assay. However, the increase amount of BUN urea is involved in such disorders like kidney failure, congestive cardiac failure, dehydration, shock, digestive system bleeding, acute heart attack, stress, high protein consumes and protein catabolism.

The decrease amount of BUN urea in liver injury (acute liver disease such as hepatitis and drug toxicity), acromegaly, mal nutrition, anabolic steroids consumes, intravenous nutrition, celiac, nephrotic syndrome and inappropriate antidiuretic hormone ADH release syndrome or SIADH well observed.

Principle of the Method:

This method is based on urea in the sample hydrolyzed with urease enzyme which release Ammonia and CO2. Furthermore, ammonia in the present of GLDH, alpha ketoglutarate and NADH create a reaction to produce glutamate and NAD. The decrease absorption is directly related to low concentration of NADH which related to amount of urea in the sample.

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\text{Urease}} (NH^{4+})_2 + CO_2$$

$$NH^{4+} + \alpha\text{-Ketoglutarate} + NADH \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

- **Reagent Preparation and Stability:**

Reagent is ready for use.
Before use, mix reagent by gently inverting each bottle.
Reagent is stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
Do not use reagents over the expiration date. If in this assay want to work with single reference so the ratio of mixture should be 4 parts from R1 and 1 part of R2.
The stability at 2-8°C for 15 days.
Do not freeze and protect from light.
R1 is transparent and colorless.
R2 transparent and light yellow color.

Waste Management:

Refer to local legal requirements for chemical disposal regulations.
Warning: Handle waste as potentially biohazardous material.
Dispose of waste according to accepted laboratory instructions and procedures.

- **Warnings and Precautions:**

For In Vitro Diagnostics Use Only.
For Professional Use Only.
Carefully read instructions for use.
In case of serious damage to the bottle or cap, resulting in product leakage or contamination, do not use the reagent pack and contact your distributor.
Take all necessary precautions required when handling laboratory reagents.
Do not use components past the expiry date stated on the Bottles.
Do not interchange caps among components as contamination may occur and compromise test results. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
Any serious incident related to the product must be reported to the manufacturer and the competent authority of the Member State where the user and/or patient is located.

- **Type of Specimen:**

Use fresh serum, non-hemolysis, plasma heparinized or urine. Do not use ammonium salt and fluoride in this assay. The stability of Urea in the serum/plasma samples at 4-25°C in 5 days and at -20°C in one year (directly freeze). The Urea stability in urine samples at 20-25°C in 2 days, at 4-8°C 5 days and at -20°C in 30 days. The urine samples should be diluted 1 to 50 by distilled water before assay the number should be multiply by 51. Avoid any contamination, freezing and DE freezing the samples.

Required but not Supplied:

General chemistry calibrator from TKS or other valid calibrators.
General chemistry control Level 1 & 2 from TKS or other valid controls.
Saline solution 0.9 % NaCl
General laboratory equipments

- **Notes:**

Carefully read instructions for use.
It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with hydrochloric acid 1 N and then thoroughly rinsed it with distilled water.
Use clean disposable pipette tips for its dispensation.
Disposable and glassware material used must be free of metals, ions and detergents. The reference of total protein is light blue. Do not use if any turbidity and dark particles see in the reference.

Performance Characteristics:

Performance results can vary with the instrument used.
Data obtained in each individual laboratory may differ from these values.
Maximum determination in this assay 400 mg/dl
LOD: 2 mg/dl
For samples with a higher concentration (400 mg/dl), dilute 1:1 with 0.9 % NaCl and re-assay. Multiply result by 2.
Precision:
Intra Assay-Within run Urea

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	40.7	0.88	2.16
2	20	130	1.02	0.78

Inter Assay-Between run Urea

Sample	n	Mean (mg/dl)	SD (mg/dl)	CV (%)
1	20	40.5	1.19	2.94
2	20	128	2.07	1.61

- **Accuracy:**

Results obtained using BIOMEDIC reagents (y) did not show systematic differences when compared with other commercial reagents (x).
Correlation coefficient (r): 0.998
Regression equation: Y = 1.575 (X) - 1.1577 mg/dl
The results of the performance characteristics depend on the analyzer used.

- **Interfering Substances:**

Bilirubin (mixed isomer)	Less than 10% interference up to 600 µmol/L Bilirubin
Lipemia	Less than 10% interference up to 5 g/l intralipid
Hemolysis	Less than 10% interference up to 5g/L Hemoglobin.

- **Reference Values:**

Serum/Plasma	
Optimal/Normal	**17-43 mg/dl**
Children 1-3 years	**11-36 mg/dl**
Children 4-13 years	**15-36 mg/dl**
Teenage 14-19 years	**18-45 mg/dl**
Women 20-50 years	**15-40 mg/dl**
Women >50 years	**21-43 mg/dl**
Men 20-50 years	**19-44 mg/dl**
Men >50 years	**18-55 mg/dl**
Urine 24h	**13-36 g/24h**

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Each laboratory should establish its own expected values. The Urea results should always be reviewed with the patient's medical examination and history.

- **Assay Procedure:**

Allow reagents to reach working temperature before using.
A proportional variation of the reaction volumes indicated does not change the result

Assay conditions:

340 nm	**Wavelengths**
37 °C	**Incubation Temperature**
1 cm	**Cuvette**

Adjust the instrument to zero with distilled water.

Control/Sample/Calibrator	Blank	
800 µl	800 µl	**R1**
10 µl	-	**Control/Sample/Calibrator**
Gently mix and incubate for 2 minutes at 37°C. Then added R2		

Control/Sample/Calibrator	Blank	
200 µl	200 µl	**R2**
Gently mix absorbance after 30 seconds A1 and 90 seconds A2. ΔA= A1- A2		

Control/Sample/Calibrator	Blank	
1000 µl	1000 µl	**Single reference**
10 µl		**Control/Sample/Calibrator**
Gently mix absorbance after 30 seconds A1 and 90 seconds A2. ΔA= A1- A2		

- **Calculations:**

Urea serum/plasma(mg/dl) = $\frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}}$ X Conc. Cal. (mg/dl)

In urine samples before assay the urine should be diluted 1 to 50 by distilled water.

Urea urine(mg/dl) = $\frac{\text{Abs. Sample}}{\text{Abs. Cal./STD}}$ x Cal.Conc. (mg/dl) x 51

In urine 24 hours

Urea (g/24h) = $\frac{\text{Urine Urea (mg/dl) x Urine Volume (ml)}}{100000}$

Conversion Units:

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)

TOSE'E KIMIA SA'ADAT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

- **References:**

1-Kaplan A. Urea. Kaplan A et al. Clin Chem the C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1257-1260 and 437 and 418.
2-Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
3-Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
4-Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
5-Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.
6-Colombo J-P (ed.). Klinisch-chemische Urindiagnostik. Rotkreuz: LABOLIFE-Verlagsgesellschaft, 1994:180.
7-Kreig M et al. J Clin Chem Clin Biochem 1986; 24:863.
8-Gruder WG, Narayanan S, Wisser H, Zawta B. List of Analytes; Preanaltical Variables. Brochure in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag, 1996.
9-Talke H. Shubert, Klin Wchersehr, (1965) 43. 174.

Symbols:

The following symbols are used in the labelling of TOSE'E KIMIA SA'ADAT systems:

IVD	In Vitro Diagnostics	Σ	Contains sufficient for <n> tests
LOT	Batch Code		Temperature limit
REF	Catalogue No.		Consult instruction for use
	Expiry Date		Caution
	Date of Manufacture		Keep dry
	Manufactured by		This way up
	Biological Risks		Keep away from sunlight

ISO 13485:2016
ISO 9001:2015

IVD

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