TRUEchemie Urea Test Kit (Urease - GLDH - Kinetic)

for the quantitative determination of Urea concentration in human serum or plasma or urine

INTENDED USE

The TRUEchemie Urea Test Kit (Urease - GLDH - Kinetic) is used for the quantitative determination of Urea concentration in human serum or plasma or urine.

INTRODUCTION

Urea is the major end product of protein nitrogen metabolism in humans. It is synthesized in liver as a by-product of the de-amination of amino acids. Its elimination in the urine represents the major route for nitrogen excretion. It constitutes the largest fraction of the non protein nitrogen component of the blood. Consequently, the circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated urea levels can occur with dietary changes, diseases that impair kidney function, liver diseases, congestive heart failure, diabetes and infections.

PRINCIPLE

Urea is hydrolyzed to ammonia and carbon dioxide by urease. Ammonia produced in this reaction reacts with α-Ketoglutarate to form Glutamate in the presence of Glutamate Dehydrogenase (GLDH). NADH is oxidized to NAD in this reaction which is measured as decrease in absorbance at 340 nm. The rate of decrease in absorbance at 340 nm is directly proportional to UREA / BUN concentration in specimen.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + Co_2$$

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

PACK SIZE

Kit size	2 x 50 ml
Cat. no.	ADX231
Kit contents	
1) Urea Reagent (R1)	2 x 40 ml
2) Urea Reagent (R2)	2 x 10 ml
3) Urea Standard (40 mg/dL)	1 x 5 ml

REAGENTS COMPOSITION

1) Working Reagent

Tris buffer	: 120 mmol/L
ADP	: 750 mmol/L
Urease	: ≥ 40 KU/L
GLDH	: ≥ 1.2 KU/L
NADH	: 1.2 mmol/L
Stabilizer	: 25 mmol/L
Preservatives	

2) Urea Standard

Urea concentration : 40 mg/dL

REAGENT PREPARATION

Ready to use reagents.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
2. Specimens should be considered infectious and handled appropriately.
3. Avoid ingestion. DO NOT PIPETTE BY MOUTH.
4. The reagent contains sodium hydroxide that is corrosive. In case of contact with skin,flush with water. For eyes, seek medical attention.
5. The disposal of the residues has to be done as per local legal regulations.

CALIBRATION

The procedures are calibrated with the standard solution which is included with each series of tests. The absorbance is used to calculate the results.

REAGENT STORAGE & STABILITY

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN COLLECTION AND STORAGE

Unhaemolysed serum / plasma.
(Do not use serum preserved with fluoride / ammonium salts)
Urine: Dilute urine 1:50 with the distilled water before analysis and multiply the obtained value by the dilution factor.
Urea will remain stable in serum for at least 1 day at room temperature (≤ 25°C), 4 -5 days at 2-8°C and 6 months when frozen (-20°C). In urine, urea will remain stable, 4-5 days when kept at 2-8°C, provided the pH value is lower than 4.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes.
2. Test tubes/rack
3. Timer
4. 37 °C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 340 nm

TEST PROCEDURE

Primary wavelength	340 nm
Temperature	37 °C

Prewarm the reagent to reaction temperature.

	Blank (µl)	Standard (µl)	Sample (µl)
Distilled water	1000	--	--
Urea Reagent (R1)	--	800	800
Urea Reagent (R2)	--	200	200
Urea Standard	--	10	--
Sample	--	--	10

Reading

Blank the Photometer with D.I Water.

Mix working reagent and standard / sample in measuring cuvette.
Read the absorbance at 30 sec. (E1) and after 60 sec., read again (E2).

Calculations: **ΔE = E1 - E2.**

Calculations

$$\frac{\Delta E \text{ Sample}}{\Delta E \text{ Standard}} \times 40 = \text{mg of urea / dL}$$

SI Units: (mg/dl) x 0.1665 = mmol/L

Conversion

To express Urea results as BUN, divide the Urea value by 2.14

QUALITY CONTROL

Quality Controls are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUE

Serum: 10 - 50 mg/dL. Serum: 5 - 24 mg BUN / dL
Urine: 20 - 35 g/24 h. Urine: 9 - 16 gm BUN / dL
It is strongly recommended that each laboratory establish its own normal range

PERFORMANCE CHARACTERISTICS

Sensitivity: 1.2 mg/dL.
Linearity: 300 mg/dL under the described assay conditions. If the concentration is greater than linearity (300 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION:

Intra-assay precision within run (n=10)	Mean (mg/dL)	SD (mg/dL)	CV (%)
Control Level - 1	31.1	0.4	1.4
Control Level - 2	102.0	0.6	0.6

Inter-assay precision run to run (n=12)	Mean (mg/dL)	SD (mg/dL)	CV (%)
Control Level - 1	32.4	1.4	4.3
Control Level - 2	98.8	0.6	0.6

The reagent was tested for 12 days, using two different Urea concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie Urea reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.99 and the regression equation is y=0.991x+0.493. The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Fluoride as well as ammonium heparinate inhibit the reaction.
Serum samples should be free from hemolysis and turbidity

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEM PARAMETERS

Mode	:	Fixed kinetic time / Initial rate
Std. conc.	:	40
Wave length	:	340 nm
Units	:	mg/dL
Flow cell temp.	:	37 °C
Blank	:	Distilled water
Reagent (R1) volume	:	800 µL
Reagent (R2) volume	:	200 µL
Sample volume	:	10 µL
Lag / Delay time	:	30 Sec.
Read time (Fixed time)	:	60 Sec.
Low normal	:	10.00
High normal	:	50.00
Sensitivity	:	1.2
Linearity	:	300
Reaction Slope	:	Decreasing

REFERENCES

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4. Hellen,C.J & Cook,J.G.H.,(1971).,Clinical Chem. Acta., 35:33
5. Bretaudibiere, J P., Phung, H.J., Baily, M., (1976).,Clin Chem., 22: 1614
6. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use		REF Catalogue number		Caution	
IVD *In vitro* diagnostic medical device		LOT Batch code		Non-sterile	
2°C 8°C Temperature limit 2-8°C		Do not re-use		Use-by date	
Manufacturer		Date of manufacture		Keep dry	

Do not use if package is damaged — Keep away from sunlight

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