Urea

CYPRESS DIAGNOSTICS

CE

IVD

Urease-GLDH. UV. Kinetic
Liquid

Store at 2 - 8 °C

Configuration

REF	HBL030	HBL030M
VOL	240 + 60 mL	6 x 30 + 3 x 15 mL
Reagent 1	1 x 240 mL	6 x 30 mL
Reagent 2	1 x 60 mL	3 x 15 mL
Standard	1 x 5 mL	-

Intended use
The Cypress Diagnostics Urea kit is an *in vitro* diagnostic medical device intended to be used by healthcare professionals for the quantitative measurement of urea in human serum or plasma.
The measurement of urea is intended to be used for the evaluation of kidney function as part of a routine health check-up (screening), as an aid in diagnosis of kidney disease and for the monitoring of the effectiveness of dialysis.
For *in vitro* diagnostic use only. For professional use only.

Clinical significance
Urea is the final result of the metabolism of proteins; it is formed in the liver from its destruction. Elevated urea levels can appear in blood (uremia) in diets with excess of proteins, renal disease, heart failure, gastrointestinal hemorrhage, dehydration or renal obstruction.[1,4,5]
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle
Urea is hydrolyzed enzymatically into ammonia (NH_4^+) and CO_3^{2-} (carbonate). NH_4^+ reacts with α-ketoglutarate to form L-glutamate in a reaction catalyzed by glutamate dehydrogenase (GLDH) with simultaneous oxidation of NADH to NAD+.

$$Urea + 2H_2O \xrightarrow{Urease} CO_3^{2-} + 2NH_4^+$$

$$NH_4^+ + \alpha\text{-}ketoglutarate + NADH \xrightarrow{GLDH} H_2O + NAD^+ + L\text{-}glutamate$$

The decrease in concentration of NADH is proportional to the urea concentration in the sample.[1]

Reagent composition

Reagent 1	**Buffer** Tris buffer pH 7,4 (125 mmol/L) α-ketoglutarate (7,5 mmol/L) Urease (12000 U/L) GLDH (2000 U/L
Reagent 2	**Substrate** NADH (1,5 mmol/L)
Standard	Urea aqueous (see value on label)

Precautions
- Reagent 1: Warning. H290: May be corrosive to metals. P234: Keep only in original container.
- All body fluid samples should be considered potentially infectious materials and the appropriate precautions should be taken. Wear personal protective equipment such as gloves, safety glasses, lab coats or aprons when working with possible biohazard contaminants.
- Use Good Laboratory Practices (GLP) when handling this product.
- Please refer to the MSDS, available on our website, for further information.

Preparation
Working reagent: Mix 4 volumes of R1 (Buffer) with 1 volume of R2 (Substrate). After mixing, allow to stand for 30 minutes prior to use. The working reagent can be stored at 2 – 8 °C or at room temperature (15 – 25 °C), and can be used as long as the blank absorbance is > 1,00 AU. The stability of the working reagent is at least 24h at 15 - 25 °C. The standard is ready to use.

Storage, stability and disposal
All the components of the kit are stable up to the date of expiration as specified on the label, when stored tightly closed, protected from light and contaminations prevented during their use. Storage temperature for this kit is 2 - 8 °C.
Handle standard very carefully to prevent contamination. The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 340 nm ≤ 1,00 AU, the reagent should be discarded.
Do not use the product if deterioration or contamination is suspected or beyond the expiration date or open container stability period. Dispose unused or deteriorated product and waste in compliance with local regulations.

Additional material required but not provided
- Spectrophotometer or colorimeter. Minimum analyzer specifications:
  Measuring at 340 nm
  Linear measuring range: 0 - 2 AU
- Cuvettes, matching the analyzer used (1,0 cm light path)
- General laboratory equipment

Samples[1]
Serum or plasma: do not use ammonium salts or fluoride as anticoagulants.
Stability of samples: 7 days at 4 - 25 °C.

Procedure
Make sure the reagents and samples are at room temperature.
1. Wavelength 340 nm; Temperature 37 °C; Cuvette (1 cm light path).
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

For Blank	1,00 mL Working reagent (R1 + R2)
For Standard Note 3	10 µL Standard + 1,00 mL Working reagent (R1 + R2)
For Sample	10 µL Sample + 1,00 mL Working reagent (R1 + R2)
Mix and start stopwatch. Read absorbance 1 (A1) after 30 sec. and A2 after 90 sec.	

Calculation
Calculate ΔA = (A2 - A1)

$$Urea\ (mg/dL) = \frac{\Delta A_{Sample}}{\Delta A_{Standard}} x\ stand.conc.\ (mg/dL)$$

Conversion Factor:
Urea mmol/L = 0,1665 x mg/dL
BUN [mg/dL] = Urea [mg/dL]/2,14 = Urea [mmol/L]/0,357

Quality control
Control sera are recommended for the qualification of the reagents and to monitor the performance of assay procedures. Next to legal requirements, we recommend to perform Quality Control on a daily basis and after each calibration.
Use Biochemistry Normal and Pathological Controls (HBC01, HBC02). If other controls (not manufactured by Cypress Diagnostics) are used, they have to be validated by the user as they can vary. Prepare and measure these controls the same as samples. Measure at least one replicate per control.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems. Do not continue testing as the results will be invalid. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference values

Serum or plasma	15 - 45 mg/dL (2,5 - 7,5 mmol/L)

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance characteristics
Measuring range: from 1,63 mg/dL (detection limit) to 206 mg/dL (linearity limit). If the obtained results are greater than 206 mg/dL, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2.

Precision:

	intra-assay (n=80)			inter-assay (n=80)		
Mean (mg/dL)	17,0	50,4	164	17,0	50,4	164
SD	0,9	1,8	7	1,2	2,6	8
CV (%)	5,3	3,5	4,1	6,8	5,2	4,9

Sensitivity: 1 mg/dL = 0,0016 ΔAU

Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.
The results of the performance characteristics depend on the analyzer used.

Interferences
It is recommended to use heparin as anticoagulant. Do not use ammonium salts or fluoride.
No interferences were observed with bilirubin (up to 20 mg/dL), hemoglobin (up to 7,5 g/L) and lipids (up to 220 mg/dL). A list of drugs and other interfering substances with urea determination has been reported by Young et al.[2,3]

Notes
1. For best use of this kit on a Cypress Diagnostics analyzer, we kindly advise to follow the application sheets of the respective analyzer. Please log in to our website (www.diagnostics.be) as a registered user to download the latest application sheets, which are located under the section of the corresponding analyzer. Compatible Cypress analyzers: CYANSmart, CYANStart, CYANExpert 130, CYANVision.
2. In case other instruments (not manufactured by Cypress Diagnostics) are used, the laboratory is responsible to validate the reagents in this kit on those analyzers before testing patient samples.
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In this case, it is recommended to use a serum based Biochemistry calibrator (HBC03).
4. Glassware and distilled water must be free of ammonia and ammonium salts.
5. Use clean disposable pipette tips for its dispensation.

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be

Bibliography

1. Kaplan A. Urea. Kaplan A et al. Clin Chem The C.V. Mosby CO. St Louis. Toronto. Princeton 1984; 1257-1260 and 237 and 418.
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press 1995.
3. Young DS. Effects of diseases 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.

Notice: Any serious incident that has occurred in relation to the device shall be reported to Cypress Diagnostics and the competent authority of the Member State in which the user and/or patient is established.

2022-02, Rev. 3.0

Cypress Diagnostics: Nijverheidsstraat 8 • 2235 Hulshout • Belgium
www.diagnostics.be • Tel: ++ 32 15 67 67 68 • e-mail: cypress@diagnostics.be