



# UREA

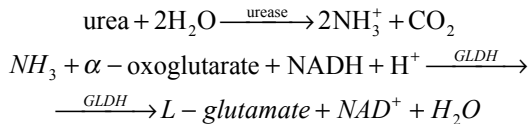
(Urease-GLDH / liquid)

Cat.No. 101-0439

Size: 4x150 ml / 4x50 ml

**PRINCIPLE:**

Urease catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. The ammonia thus produced combines with  $\alpha$ -oxoglutarate in the presence of nicotinamide adenine dinucleotide, reduced, (NADH) during the glutamate dehydrogenase (GLDH) catalysed reaction to yield glutamate. An equimolar quantity of NADH undergoes oxidation during the reaction and therefore the decrease in absorbance at 340 nm due to NADH oxidation is directly proportional to the blood urea nitrogen concentration in the sample.



**SAMPLE:**

Serum, plasma or urine.  
 Dilute urine 1:50 with distilled water.  
 Urea in the sample is stable 7 days at +2 to +8 °C.

**REAGENTS:**

- |                              |                            |
|------------------------------|----------------------------|
| 1. Reagent 1, Buffer         |                            |
| Tris buffer pH 7.8           | 80 mmol/L                  |
| $\alpha$ -ketoglutarate      | 6 mmol/L                   |
| Urease                       | 75000 U/L                  |
| 2. Reagent 2, Enzyme reagent |                            |
| GLDH                         | 60000 U/L                  |
| NADH                         | 0.32 mmol/L                |
| 3. Standard                  |                            |
| UREA                         | 8.3 mmol/L (50 mg/dL UREA) |

Store at +2 °C to +8 °C.

**PREPARATION OF REAGENTS:**

All reagents are ready to use.  
 This reagent is stable up to the date of expiration at +2 °C to +8 °C.  
 Preparation of working reagent: mix 3 vol R1 + 1 vol R2. The working reagent is stable for 2 months at +2 °C to +8 °C.

**PROCEDURE:**

- |              |                        |
|--------------|------------------------|
| Wavelength:  | 340 nm, 334 nm, 365 nm |
| Cuvette:     | 1 cm light path        |
| Temperature: | 25 °C, 30 °C, 37 °C    |
| Zero:        | air or distilled water |

**MONOREAGENT METHOD:**

Pipette into test tubes	Standard	Sample
Standard	30 $\mu$ l	-
Sample	-	30 $\mu$ l
Working reagent	1000 $\mu$ l	1000 $\mu$ l
Mix and read the absorbance after 30 sec (A <sub>1</sub> ) and after 90 sec read (A <sub>2</sub> ) min. Calculate $\Delta A = A_1 - A_2$ .		

**TWO REAGENTS METHOD:**

Reagent 1	900 $\mu$ l
Sample or standard	30 $\mu$ l
Wait 3 minutes. Add:	
Reagent 2	300 $\mu$ l
Mix and read the absorbance after 30 sec (A <sub>1</sub> ) and after 90 sec read (A <sub>2</sub> ) min. Calculate $\Delta A = A_1 - A_2$ .	

**CALCULATION:**

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{stand.conc.} = \text{Urea conc.}$$

Conversion factor: mg/dL x 0.1665 = mmol/L

**EXPECTED VALUES:**

Serum	2.49 – 7.49 mmol/L (15-45 mg/dL)
Urine	20-35 g/24h

**LINEARITY:**

up to 59.50 mmol/L (350 mg/dL)

**QUALITY CONTROL:**

CONTRO-N	20 x 5 ml	Cat. No. 101-0083
CONTRO-P	20 x 5 ml	Cat. No. 101-0084

**NOTES:**

- Reagents contain sodium azide as stabilizer. Do not swallow. Avoid contact with the skin and mucous membranes.
- If the urea concentration in the serum > 350 mg/dL dilute sample 1:2 with physiological solution and repeat assay (result x 2).
- Do not use hemolyzed serums.

**REFERENCES:**

- Fawcett, J.K., J.Clin.Path. 13, 15 (1960)
- Chaney, A.Clin.Chem. 8, 130 (1962)