

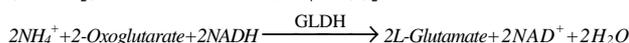
Cat. No.:	46661,46661S	46662,46662S	46663
	120 ml	500 ml	10x20 ml
	(1x90 ml+1x30ml)	(1x375 ml +1x125 ml)	(10x15 ml+ 10x5 ml)

**Reagent kit for determination of urea concentration in serum and urine. Enzymatic (UV) method.**

The detoxification of  $\text{NH}_4^+$  formed in the catabolism of amino acids takes place in the urea cycle. Enzymes catalyzing these reactions are synthesized in the liver. The end product is Carbamide (Urea) which is a nontoxic, nonpolar, small molecule. It is eliminated by the kidney. Increased levels are associated with renal diseases, as well as dehydration, circulatory collapse, gastrointestinal hemorrhage and diabetic coma. Decreased values are observed in some cases of severe liver disease.

**Principle**

Ammonia and Carbon dioxide ( $\text{CO}_2$ ) are produced when urea is hydrolyzed in presence of Urease. The Ammonia produced in the reaction combines with 2-Oxoglutarate and NADH in the presence of Glutamate dehydrogenase (GLDH) to yield glutamate and  $\text{NAD}^+$ . The NADH/ $\text{NAD}^+$  reaction produces a unique change in absorbance at 340 nm, which correlates with the concentration of urea nitrogen in the sample.



**Reference values**

**Serum:** 2.49-7.47 mmol/l (15-40 mg/dl)  
**Urine:** 333-583 mmol/24 h (20-35 g/24 h)

It is recommended that each laboratory should assign its own normal range.

**Reagents**

**1. Reagent (R1)**

NADH 320  $\mu\text{mol/l}$

**2. Reagent (R2)**

Tris buffer, pH=7.60 100 mmol/l  
 $\alpha$ -Ketoglutarate 9 mmol/l  
Urease 6500 U/l  
GLDH 1100 U/l

**3. Urea standard**

Ready for use. For details please check the insert.  
Available only in Cat. No.: 46661S and 46662S

**Avoid direct exposure to light.**

**Precaution**

These reagents contain 0.1 % sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted reagent.

**Sample**

Serum free of haemolysis.  
Urine diluted in ratio of 1:100 with distilled water. Do not use anticoagulants containing fluoride or ammonium ions!

**PROCEDURE**

**Preparation and stability of working reagent**

- One-reagent procedure  
Mix 3 volumes of reagent (R1) with 1 volume of reagent (R2). Stability:  
at 20-25 °C: 7 days  
at 2-8 °C: 4 weeks
- Two-reagent procedure  
Reagents are ready for use.  
If the absorbance of working reagent is lower than 1.5 at 334 nm the reagent can not be used.

**Assay conditions**

Wavelength: 340 (334-365) nm  
Temperature: 37 °C  
Cuvette: 1 cm light path  
Read against: distilled water  
Method: kinetic (decreasing)

**• One-reagent procedure**

Working reagent	1,0 ml
Sample	10 $\mu\text{l}$

Mix and after 30 seconds incubation measure the absorbance during 1 minute.

**• Two-reagent procedure**

R1	1,5 ml
Sample	20 $\mu\text{l}$

Mix and after 60 seconds incubation then add:

R2	500 $\mu\text{l}$
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Mix and after 30 seconds incubation measure the absorbance during 1 minute.

**Calibration (37°C, UV method)**

- S1: Distilled water
- S2: Urea standard Cat. No.: 50811 or Roche C.F.A.S. (Calibrator for automated system) or Randox Calibration Serum Level I or Randox Calibration Serum Level II

**Calibration frequency**

Two-point calibration is recommended:  
- after reagent lot change,  
- as required following quality control procedures.

**Calculation using calibration**

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}$$

A = Absorbance, C = Concentration

**Quality control**

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**PERFORMANCE DATA**

The following data were obtained using the Olympus 600 analyser (37°C).

**Linearity**

The test is linear up to 66.7 mmol/l (400 mg/dl).

**Sensitivity**

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 0,009 mmol/l (0,05 mg/dl) Urea concentration at 334 nm.

**Precision**

Sample	Reproducibility		
	Average concentration (mmol/l)	SD	CV%
Sample I.	7.6	0.25	3.26
Sample II.	25.6	0.90	3.51

**Repeatability (twenty replicates)**

Sample	Repeatability (twenty replicates)		
	Average concentration (mmol/l)	SD	CV%
Sample I.	7.4	0.12	1.58
Sample II.	23.1	0.30	1.29

**Correlation**

Comparative studies were done to compare our reagent with our Urea UV powder reagent.  
The results from these studies are detailed below.  
Correlation coefficient:  $r = 0.9994$   
Linear regression:  $y \text{ (mmol/l)} = 0.972x + 0.710$   
(x= powder reagent, y= liquid reagent).

**Specificity**

Bilirubin 855  $\mu\text{mol/l}$  (50mg/dl), lipid 1000 mg/dl, glucose 55.5 mmol/l (1000mg/dl) and ascorbic acid 2.84 mmol/l (50mg/dl) don't interfere with the assay at the given levels.

**Note**

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

**For in vitro diagnostic use only.**

**The following symbols are used on labels**

 For in vitro diagnostic use

 Use by (last day of the month)

 Temperature limitation

 Batch Code

 Code

**Bibliography**

Talke H., Schubert G.E. Klin.1965, 43:174.