



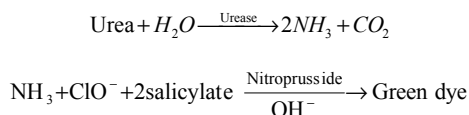
# BUN

## Enzymatic Colorimetric Method ( Berthelot mod.)

Cat.No. 101-0248      Size 4 x 250 ml  
 Cat.No. 101-0364      Size 6 x 100 ml  
 Cat.No. 101-0425      Size 2 x 100 ml

### PRINCIPLE:

Urea is hydrolyzed with urease to produce ammonia and carbon dioxide. In a modified Berthelot reaction the ammonium ions react with hypochlorite and salicylate to give a green dye. The increase of absorbance at 600 nm is proportional to the urea concentration in the sample.



### SAMPLE:

Serum, heparinized or EDTA plasma, or urine. Dilute urine 1:50 with distilled water.  
 Stability 3 days at +2 °C to +8 °C.

### REAGENTS:

- Reagent 1  
 Phosphate buffer pH=6.7      50 mmol/L  
 Sodium salicylate      60 mmol/L  
 Sodium nitroprusside      3.2 mmol/L  
 EDTA      2 mmol/L
- Reagent 2  
 Sodium hydroxide      150 mmol/L  
 Sodium hypochlorite      140 mmol/L
- Reagent 3  
 Urease      30000 U/L
- Standard  
 BUN      Standard concentration see on the vial label

Store the reagents at +2 °C to +8 °C.

### REAGENT PREPARATION:

Reagent 1 and Reagent 3 (Working solution):  
 Dissolve contents of one vial Reagent 3 with Reagent 1, shaking gently. The reconstituted reagent is stable 4 weeks at +2 °C to +8 °C or 1 week at room temperature.

Reagent 2 is ready for use.

### PROCEDURE:

Method:      End Point  
 Wavelength:      580 nm  
 Cuvette:      1 cm light path  
 Temperature:      room temperature; 37 °C  
 Color stability:      30 min  
 Zero:      reagent blank

Pipette into test tubes:	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Working sol.	1 ml	1 ml	1 ml
Mix, incubate 10 min. at room temperature or 5 min. at 37 °C.			
Reagent 2	1 ml	1 ml	1 ml
Mix, incubate 10 min. at room temperature or 5 min. at 37 °C. Read absorbance of sample and standard against reagent blank.			

### NOTE:

Volumes can be proportionally changed.

### CALCULATION:

$$\frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{conc. standard} = \text{mmol/L BUN}$$

### EXPECTED VALUES:

Serum, plasma:	1.7- 8.3 mmol/L N-urea
	4.8-23.3 mg/dl N-urea
Urine:	333-600 mmol/24 h N-urea
	9.4-18.9 g/24 h N-urea

### LINEARITY

up to 33.3 mmol/L (93.5 mg/dl N-Urea)

### QUALITY CONTROL:

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

### NOTE:

- Stability: until expiration date on label.
- Interfering substances: ammonium ions and fluoride.
- All anticoagulants except ammonium heparinate can be used.
- Reagent 2 contains sodium hydroxide and hypochlorite: avoid contact with skin and mucous membranes.
- Do not use lipemic, icteric and haemolytic serum or plasma samples.

### REFERENCES:

- Berthelot M.: Report Chem.Aplique 1, 284 (1859).
- Fawcet J.K.; Scott J.E.: J.Clin.Path. 13, 156 (1960).
- Balleter, WG. Bushman, C.J. Tidwell, P.W. Anal. Chem. 33, 592 (1961).
- Watherburn, M.W., Anal. Chem., 39, 971 (1967).