

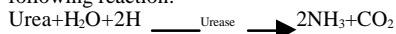
UREA COLORIMETRIC

ENDPOINT DETERMINATION

UREASE- BERTHELOT REACTION

Principle:

Enzymatic determination of Urea according to the following reaction:



The ammonium ions formed react with salicylate and hypochlorite to give a green dye (2,2-Dicarboxylindophenol)

Reagent Concentration:

Buffer:

Phosphate buffer, pH6.7	50 mmol/l
EDTA	2mmol/l
Sodium salicylate	60mmol/l
Sodium nitroprusiate	3.2mmol/l

Enzyme reagent:

Urease 30000U/l

Hypochlorite solution:

Sodium hypochloride	140mmol/l
Sodium hydroxide	150mmol/l

Standard:

Urea 50mg/dl (8.325mmol/l)

Preparation and Stability:

Buffer /R1 is ready to use. Add 1 vial enzyme reagent /R2 to 1 bottle of buffer /R1. The working solution is stable for 4 weeks at +2 to +8°C and 6 days at +20 to +25°C. Reagent /R3 and Standard /R4 are ready to use and stable up to the expiry date specified when stored at +2 to +8°C. Protect all reagents against direct light!

Samples:

Sera, heparinised serum, citrated plasma. Urine diluted 1:50 with distilled water.

Procedure:

Wavelength:	580nm
Temperature:	+25 / +37°C
Cuvette:	1cm light path
Zero adjustment:	Against reagent blank

	Reagent blank	Standard	Sample
Working Solution (R1/R2)	1000µl	1000µl	1000µl
Standard /R4	---	10µl	---
Sample	---	---	10µl

Mix and incubate at +37°C for 5 minutes or for 10 minutes at +15 to +25°C. Then add:

Reagent /R3	1000µl	1000µl	1000µl
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Mix and incubate at +37°C for 5 minutes or at +15 to +25°C for 10 minutes.

Calculation:

? A sample
----- x standard conc. = Urea con. in mg/dl
? A standard
Standard concentration: 50mg/dl (8.325mmol/l)

Linearity:

Up to 200mg/dl (33.3mmol/l) for the normal pipetting scheme. The linearity using the diluted reagent /R3 is enhanced up to 300mg/dl (50mmol/l)
In case of higher results, dilute sample 1:2 with saline solution and repeat test. Multiply result by 2.

Normal Values:

Serum	15-45mg/dl (1.7-8.3mmol/l) Urea 4.7-23mg/100ml Urea-N.
Urine 24 hours	20-36g (333-600mmol) Urea 9.4-16.9g Urea-N

The normal values are influenced by daily take-up of proteins in relation to the body weight.

Notes:

Use glassware free of ammonium ions.

Quality Control:

For accuracy and reproducibility control:-
Assayed Multi-Sera Normal and Elevated.
For reproducibility control:-
Multi-Sera Loaw, Normal and Elevated.

Presentation:

#URE0380 2 x 125ml, 125 tests

Buffer	1 x 125ml
Enzyme reagent	1 x 125ml
Hypochlorite solution	1 x 125ml
Standard	1 x 5ml

Safety precautions:

For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Health and Safety data sheets are available on request.

Literature:

Berthelot, M. P. E., Report Chim. Appl., 384, (1859)
Patton, C. J., Crouch, S. R., Anal. Chem., 49, 464-469 (1977)
Fawcett, J.K. and Scott, J.E., J. Clin Path., 13, 156 (1960)
Weatherburn, M.W., Anal. Chem., 39, 971 (1967)