

UREA – GLDH (DST)

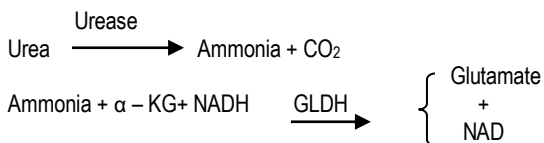
(UV – FIX TIME)

Reagent kit for quantitative estimation of UREA/BUN in serum or plasma.

1. Reagent formulation to the convenience of customer.
 - (a) Liquid Reagent also available.
 - (b) Two reagent system with long reconstituted stability.
2. A very good linearity.
3. Kinetic two readings with result in 2 minutes.

PRINCIPLE:

Urea is acted upon by urease releasing ammonia and carbon dioxide. The ammonia generated is utilised by glutamate dehydrogenase (GLDH) in the presence of 2-Oxoglutarate (α KG) to form glutamate. Simultaneously converting NADH to NAD resulting in a decrease in absorbance at 340 nm.



The rate of decrease in absorbance per minute is measured at 340 nm.

DIAGNOSTIC SIGNIFICANCE:

Increased urea level can occur in liver disease, congestive heart failure, diabetes, infections, in diseases which impair kidney function and with dietary changes. It is also increased in adrenocortical insufficiency, acute intestinal occlusion, various poisonings, shock, urine retention and raised protein break down.

Decreased levels are seen in malnutrition, hepatic failure and pregnancy.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Buffer	100 mmol/L
* α – KG	10 mmol/L
* ADP Na ₂	0.5 mmol/L
* Urease	5000 U/L
* GLDH	1000 U/L
* NADH Na ₂	0.1 mmol/L
pH 7.9 ± 0.5 at 25°C	
Reagent-2	
Buffer	100 mmol/L

Urea Standard (50 mg/dl)

Also contains non-reactive fillers and Stabilizers.

PRESENTATION :

	No. of Bottles			
	5x20 ml	2x50 ml	4x50 ml	10x50 ml
All reagents to be stored at 2-8°C				
1 UREA (Enzyme/Co-enzyme)	5	2	4	10
2 UREA (Buffer)	5	2	4	10
Urea standard (50 mg/dl)	1	1	1	2

PRECAUTION:

ENZOPAK UREA/BUN is for *IN-VITRO* diagnostic use only.

Reagent contains Sodium Azide, DO NOT INGEST.

PREPARATION OF WORKING REAGENT

FOR 5 x 20 ml

Carefully transfer the content of 1 Urea (Powder) into the bottle containing 20 ml of 2 Urea Buffer. Mix well to dissolve. Wait for 15 minutes before use.

FOR 2 x 50 ml, 4 x 50 ml and 10 x 50 ml

Carefully transfer the content of 1 Urea (Powder) to the bottle containing 50 ml of 2 Urea Buffer. Mix well to dissolve. Wait for 15 minutes before use.

REAGENT STORAGE AND STABILITY:

ENZOPAK Urea reagents are stable at 2-8° C. until the expiry date stated on the label.

Working reagent is stable for 4 months at 2-8° C.

SPECIMEN COLLECTION:

Fresh, fasting, clear serum with no hemolysis is the specimen of choice. Plasma, collected using heparin, oxalate or citrate as an anticoagulant, may also be used.

GENERAL PROCEDURE:

- Type of Reaction : Fix Time / Decreasing OD
- Wavelength : 340 nm
- Flowcell Temperature : 25°C / 30°C
- Sample Volume : 20 μ l (0.02 ml.)
- Working Reagent Volume : 1.0 ml.
- Initial Delay : 30 or 20 Seconds
- Reaction Time : 60 Seconds
- Light Path : 1.0 cm.
- Zero setting with : Distilled Water

PROCEDURE:

For laboratories using instruments with 1.0 ml. cuvette capacity.

PIPETTE IN TO TEST TUBES	STD.	TEST
• WORKING REAGENT (ml)	1.0	1.0
• STANDARD (ml)	0.02	-
• SAMPLE (ml)	-	0.02

Mix immediately and read difference in absorbance between 20 seconds (AT₁) and 80 seconds (AT₂) or between 30 Seconds (AT₁) and 90 seconds (AT₂) for standard and test.

TEST RESULTS:

Urea (mg/dl) = $\Delta A/\text{minute} \times \text{Factor}$

Where $\Delta A/\text{minute} = (AT_1 - AT_2)$

and Factor = $\frac{\text{Concentration of Std. (mg/dl)}}{\Delta A/\text{min of Std.}}$

= $\frac{50}{\Delta A/\text{min of Std.}}$

NORMAL VALUES:

Serum : 10 to 45 mg/dl

Serum BUN : 5 to 21 mg/dl

LINEARITY:

The method is linear upto 300 mg/dl. For Urea concentration higher than linearity limit, mix one volume of sample with one volume of 0.9 % saline and multiply the results obtained by two.

REFERENCE:

1. Talke H., Schubert G. E., Klin. Wschr... 43, (1965), 174



