UREA

ENZOPAK
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(Berthelot Method)

PRODUCT HIGHLIGHTS

- Reconstitution of enzyme reagent with Deionised Water
- Linearity up to 350 mg/dl
- Ready to use color reagent

BACKGROUND & SYNOPSIS:

In 1993, Marshall devised a method for estimation of urea consisting of hydrolysis by urease, followed by titrimetric estimation of ammonia. A gasometric method was established by Van Slyke in 1914 on a similar principle of hydrolysis of urea. Colorimetric estimation of ammonia was made possible by Nessler's reagent. But, this method involved deproteinization and posed turbidity problems at higher levels of urea concentrations. Subsequently, coupling of urease method with Berthelot reaction eliminated these problems and increased sensitivity many folds.

Earlier versions of Urease-Berthelot reaction used four reagents. In 1962, Chaney and Marbach modified the method by combining reagents to make it a three reagent system and simplified the technique. Use of sodium salicylate instead of phenol and the use of sodium nitroprusside as an accelerator has improved performance of the reagent system making it a two reagent system.

ENZOPAK Urea is formulated on two reagent system and offers the advantages of a simple, enzymatic, sensitive and specific method.

PRINCIPLE:

Urease breaks down urea into ammonia and carbon dioxide. In alkaline medium, ammonia reacts with hypochlorite and salicylate to form dicarboxyindophenol, a coloured compound. The reaction is catalysed by sodium nitroprusside. The intensity of colour produced is measured photometrically at 578 nm (570-620 nm)

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} \text{Ammonia} + CO_2$$

$$\left.\begin{matrix}\text{Ammonia} + \text{Salicylate} \\ + \\ \text{Hypochlorite}\end{matrix}\right\} \xrightarrow{\text{Nitroprusside}} \text{2-2 Dicarboxy Indophenol}$$

DIAGNOSTIC SIGNIFICANCE:

Increased urea levels can occur in liver diseases, congestive heart failure, diabetes, infections and in diseases which impair kidney functions. It is also increased in adrenocortical insufficiency, acute intestinal occlusion, various poisonings, shocks, urine retension and raised protein break down. Decreased levels are seen in malnutrition, hepatic failure & pregnancy.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
• Buffer	100 mmol/L
• Urease	≥ 10000 U/L
• Sodium Nitroprusside	2 mmol/L
• Sodium Salicylate	40 mmol/L
pH 6.8± 0.5 at 25°C	
Reagent-2	
• Sodium Hypochlorite	8 mmol/L
Urea Standard (40 mg/dl)	

Also contains non-reactive fillers and Stabilizers.

PRESENTATION :

All reagents to be stored at 2-8°C

	No. of Bottles / Vials		
	5x10 ml	2x50 ml	6x50 ml
* 1 Urea (Buffered Enzyme)	5	2	6
* 2 Urea (Color Developing Reagent) (Ready to use)	1	2	3
* Urea Standard (40 mg/dl)	1	1	1
* Bottle for Reconstitution	1	1	1

PRECAUTION:

ENZOPAK UREA/BUN is for *in-vitro* diagnostic use only.
Reagent contains Sodium Azide. DO NOT INGEST.

PREPARATION OF WORKING REAGENT:
(USE GOOD QUALITY DEIONISED WATER)

1 Urea (Buffered Enzyme): for 5x10 ml

Dissolve the contents of 1 Urea (Enzyme) with 10 ml of Deionised water. Mix well and wait for 10 minutes before use.

1 Urea (Buffered Enzyme): for 2x50 ml & 6x50 ml

Dissolve the contents of 1 Urea (Enzyme) with 50 ml of Deionised water. Mix well and wait for 10 minutes before use.

REAGENT 2 Urea (Color Developing Reagent):

Ready to Use

Store at 2-8°C in a tightly capped container.
2 Urea: Corrosive! Do not pipette by mouth.

REAGENT STORAGE AND STABILITY:

Working Enzyme Reagent after reconstitution is stable for 6 months when stored at 2-8°C.Protect the working reagent from light.

2 Urea Color Developing Reagent once opened is stable for 3 months when stored at 2-8°C.

All reagents are stable until the expiry date stated on the kit label.

SPECIMEN COLLECTION:

Fresh serum, plasma with anticoagulant – heparin, oxalate or citrate.
Urine diluted 1:99 in normal saline (Result multiply by 100).

REACTION PARAMETERS:

* Type of Reaction	: End Point
* Wavelength	: 578 nm (570 - 620)
* Flowcell Temperature	: 37°C
* Incubation	: 5 min + 5 min. at 37°C
* Std. Concentration	: 40 mg/dl
* Sample volume	: 10 µl (0.01 ml)
* Reagent volume R1+R2	: 1.0 ml + 1.0 ml
* Light Path	: 1.0 cm
* Zero setting with	: Reagent blank

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PROCEDURE:

PIPETTE INTO	Procedure for 2.0 ml.			Procedure for 1.0 ml.		
TEST TUBES	BLK	STD.	TEST	BLK	STD.	TEST
• STD. (ml)	-	0.01	-	-	0.005	-
• SAMPLE (ml)	-	-	0.01	-	-	0.005
• Distilled water (ml)	0.01	-	-	0.005	-	-
• Working Reagent (ml)	1.0	1.0	1.0	0.50	0.50	0.50

Mix well and incubate for ten minutes at room temperature (25-30°C) or five minutes at 37°C.

Color Developing Reagent (ml)	1.0	1.0	1.0	0.50	0.50	0.50

Mix well and incubate for ten minutes at room temperature (25-30°C) or five minutes at 37°C. Read absorbance of test and standard against reagent blank at 578 nm (570-620 nm or with RED filter).

STABILITY OF REACTION MIXTURE:

The color of final reaction mixture is stable for one hour.

CALCULATION:

$$\text{Urea Concentration (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std.}} \times 40$$

NORMAL VALUES:

Serum Urea : 10 to 45 mg/dl (1.7 to 7.5 mmol/lit).
Serum BUN : 5 to 21 mg/dl
Urine Urea : 20 -30 gm / 24 hrs.

BUN Concentration (mg/dl) = 0.467 x Urea Concentration (mg/dl)

To convert Urea concentration (mg/dl) to mmol/lit., use the following equation.

Urea concentration (mg/dl) x 0.167 = mmol/lit.

LINEARITY:

The method is linear upto 350 mg/dl. For Urea concentration higher than linearity limit, mix one volume of sample with one volume of 0.9 % saline and repeat the assay. Multiply the results obtained by two.

PROCEDURE FOR ESTIMATION OF UREA IN URINE:

Dilute the sample 1:50 with distilled water, follow the procedure given for serum urea estimation and calculate the test results as follows.

$$\text{Urea conc. (gms/liter)} = \frac{\text{Abs. of test}}{\text{Abs. of Std.}} \times 40 \times \text{dilution factor} \times \frac{1}{100}$$

LIMITATIONS :

1. Storage condition as mentioned on the kit to be adhered.
2. Do not freeze or expose reagent to high temperature and protect from direct sunlight as it may affect the performance of the kit.
3. The chromogen reagent 2 being a super saturated solution may tend towards forming crystals. 5 mins incubation at 37°C would dissolve the crystals.
4. Before the assay bring all the reagents to room temperature.
5. Avoid contamination of the reagents during the assay process.
6. Use clean glassware free from dust or debris.

REFERENCES:

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