ENZOPAK

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URIC ACID (DST)

(URICASE METHOD)
Reagent kit for quantitative estimation of Uric acid in serum or plasma.

PRODUCT HIGHLIGHTS :

- **Reagent free from interference due to reducing substances**
- **Specially related chromogen for increased sensitivity.**

BACKGROUND AND SYNOPSIS :

Uric acid is produced by the action of xanthine oxidase on xanthine and hypoxanthine, which are products of nucleic acid degradation. The chemical method of estimation of uric acid is based on the uric acid forming a blue phosphotungstate complex. However, this method in serum or plasma continue to produce colour by phosphotungstate complex. In 1980, Fossati et, al described a modified Trinder method for the assay of uric acid.
ENZOPAK Uric Acid is formulated on this method with the advantage that the method is specific and sensitive.

PRINCIPLE :

Uricase is a very specific enzyme acting on Uric Acid, end products being allantoin and peroxide. Peroxidase is used to utilize hydrogen peroxide (proportional to uric acid concentration) to convert chromogen to coloured complex. The intensity of colour produced is proportional to uric acid concentration and is measured photometrically at 520 nm (500-550 nm or GREEN filter).

$$\text{Uric Acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{TBHB} \xrightarrow{\text{Peroxidase}} \text{coloured complex}$$

TBHB :2,4,6-Tribromo-3-hydroxy benzoic acid.

DIAGNOSTIC SIGNIFICANCE :

Uric Acid levels in any subject may vary during a day from time to time and from one to another day. The changes represent the contents in diet and metabolism besides pathological condition.
The diagnostic values showing increased uric acid levels are in kidneys failure and gout. Elevated levels are found in acute infectious diseases, severe uremia, toxemia of pregnancy, leukemia and some malignant diseases or conditions.
The decreased levels are found due to drugs and hormone treatment like adrenocorticotropic hormone (ACTH).

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
• Uricase	≥ 200 U/L
• POD	≥ 1500 U/L
• 4 – AAP	0.2 mmol/L
• TBHB	0.5 mmol/L
Reagent-2	
* Buffer	100 mmol/L
* Detergent	1 mmol/L

pH 8.2 ± 0.5 at 25^0 C
Uric Acid Standard (6mg/dl)
Also contains non-reactive fillers and Stabilizers.

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PRESENTATION :

	NO. of Bottles/Vials		
All reagents to be stored at 2-8^{0}C	5x10 ml	5x20 ml	4x50 ml
* 1 Uric Acid (Enzymes, Chromogen)	5	5	4
* 2 Uric Acid (Buffer)	1	5	4
* Uric Acid Standard (6 mg/dl)	1	1	1

For 5x10 ml reconstitution bottle provided

PRECAUTION :

ENZOPAK Uric acid is for *IN-VITRO* diagnostic use only.
Reagent contains Sodium Azide. DO NOT INGEST.

PREPARATION OF WORKING REAGENT :

FOR 5 x 10 ml.
Carefully transfer the content of 1 Uric Acid (Powder) into the bottle containing 10ml of 2 Uric Acid Buffer. Mix well to dissolve. Wait for 5 minutes before use.

FOR 5 x 20 ml.
Carefully transfer the content of 1 Uric Acid (Powder) into the bottle containing 20ml of 2 Uric Acid Buffer. Mix well to dissolve. Wait for 5 minutes before use.

FOR 4 x 50 ml.
Carefully transfer the content of 1 Uric Acid (Powder) into the bottle containing 50 ml of 2 Uric Acid buffer. Mix well to dissolve. Wait for 5 minutes before use.

REAGENT STORAGE & STABILITY :

All reagents included in the kit are stable at 2-8^{0}C until the expiry date indicated on the label. Working reagent is stable for 6 months at 2-8^{0}C. A slight rise in the blank reading due to stored life will not affect the test results.

SPECIMEN COLLECTION :

Fresh, clear fasting serum with no hemolysis is the specimen of choice. However, plasma collected from blood using heparin as an anticoagulant may be used.

REACTION PARAMETERS :

- Type of Reaction : End Point
- Wavelength : 520 nm (500-550 nm)
- Flowcell Temperature : 37^0 C
- Incubation : 5 min. at 37 ^{0}C
- Std. Concentration : 6 mg/dL
- Sample Volume : 20 µl (0.02 ml)
- Reagent Volume : 1.0 ml.
- Light Path : 1.0 cm.
- Zero setting with : Reagent Blank

PROCEDURE :

For instruments with 1.0 ml/0.5 ml cuvette capacity or with flow cells.

PIPETTE INTO		Procedure for 1 ml.			Procedure for 0.5 ml.		
TEST TUBES		BLK	STD.	TEST	BLK	STD.	TEST
WORKING REAGENT	(ml)	1.0	1.0	1.0	0.5	0.5	0.5
STANDARD	(ml)	-	0.02	-	-	0.01	-
SAMPLE	(ml)	-	-	0.02	-	-	0.01

Mix and incubate at 37^0 C for 5 minutes and read absorbance of test and standard against reagent blank at 520 nm (500-550 nm or GREEN filter).

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PROCEDURE FOR COLORIMETERS (2.5 ml)

	PIPETTE INTO TEST TUBES	BLANK	STD.	TEST
•	WORKING REAGENT (ml)	1.0	1.0	1.0
•	STANDARD (ml)	-	0.05	-
•	SAMPLE (ml)	-	-	0.05

Mix well and incubate at 37^0C for 5 minutes.

•	Dist. Water (ml.)	1.5	1.5	1.5

Mix and read absorbance of test and standard against reagent blank with GREEN filter.

STABILITY OF FINAL COLOUR DEVELOPED :
The colour of reaction mixture is stable for one hour when protected from direct light.

TEST RESULTS :

$$\text{Uric Acid concentration (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Std.}} \times 6$$

To convert Uric Acid concentration (mg/dl) to micromoles/liter use the following equation.
Micromoles/lit. = mg/dl x 59.5

NORMAL VALUES:
Male : 3.2-7.0 mg/dl (190.4 – 416.5 µmol/l)
Female : 2.6-6.0 mg/dl (154.7 – 357 µmol/l)

LINEARITY :
This method is linear upto 20 mg/dl. For sample values higher than linearity limit, dilute the samples suitably with 0.9 % saline and repeat the assay. Apply proper dilution factor to calculate the test results by applying dilution factors.

REFERENCES :

TRIVEDI, R.C., REBBAR, L.BERKA, E., AND Strong, I: Clin. Chem 24:1908 (1978).

FOSSATI, P., PRINCIPLE, L., BERTI, G:Clin Chem 26, 227-231 (1980).

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