URIC ACID

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

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 is not specific as other reducing substances present 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 hydrogen peroxide. Peroxidase is used to utilise hydrogen peroxide (proportional to uric acid concentration) to convert chromogens to coloured complex. The intensity of colour produced is proportional to uric acid concentration and is measured photometrically at 546 nm (530-570 nm or with GREEN filter).

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

$$H_2O_2 + \text{4-Aminontipyrine} + \text{DCHBS} \xrightarrow{\text{Peroxidase}} \text{Coloured complex}$$

DIAGNOSTIC SIGNIFICANCE:
Serum uric acid levels are very labile i.e. show changes in the same person. The changes may be day-to-day or seasonal, showing purine content of diet.

The diagnostic values showing increase in 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	
• Buffer	100 mmol/L
• Urease	≥ 250 U/L
• POD	≥ 500 U/L
• 4 – AAP	0.2 mmol/L
pH 7.2 ± 0.5 at 25^0 C	
Reagent-2	
* Buffer	2 mmol/L
Uric Acid Standard (6 mg/dl)	

Also contains non-reactive fillers and Stabilizers.

PRESENTATION:

	No. of Bottles
All reagents to be stored at 2-8^0 C	15 x 1.1 ml
• 1 Uric Acid (Enzymes, Chromogen)	15
• 2 Uric Acid (Buffer)	1
• Uric Acid Standard (6 mg/dL)	1

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

PREPARATION OF WORKING REAGENT:
FOR 15 x 1.1 ml
Dissolve the contents of one Vial of 1 URIC ACID with 1.1 ml of 2 URIC ACID (Buffer). Mix gently 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 2 weeks at 2-8^{0}C. A slight rise in the blank reading due to storage 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	:	546 nm (530-570)
• Flowcell Temperature	:	37 ^{0}C
• Incubation	:	10 min. at 37 ^{0}C
• Std. Concentration	:	6 mg/dL
• Sample Volume	:	50 µl (0.05 ml)
• Reagent Volume	:	1.0 ml.
• Zero setting with	:	Reagent Blank
• Light Path	:	1.0 cm.

PROCEDURE:
For laboratories using instruments with 1.0 ml/0.5 ml cuvette capacity.

PIPETTE INTO TEST TUBES	Procedure for 1 ml.			Procedure for 0.5 ml.		
	BLK	STD.	TEST	BLK	STD.	TEST
• WORK. RGT. (ml)	1.0	1.0	1.0	0.5	0.5	0.5
• STD. (ml)	-	0.05	-	-	0.025	-
• SAMPLE (ml)	-	-	0.05	-	-	0.025

Mix and incubate at 37^0 C for 10 min. and read absorbance of test and standard against reagent blank at 546 nm (530-570 nm or green filter).

Procedure for Colorimeters (2.5 ml)

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

Mix well and incubate at 37^{0}C for 10 minutes. Add...

• Dist. Water (ml.)	1.5	1.5	1.5

Mix and read absorbance of test and standard against reagent blank at 546 nm (530-570 nm or green filter).

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

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URIC ACID

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)

NORMAL VALUES (URINE) :

Urine uric acid content: 250-750 mg/24 hours.

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:

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

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