



URIC ACID (URICASE-POD Method, End Point)

Intended Use

The reagents are used for the quantitative determination of Uric acid in serum or plasma. For in-vitro diagnostic use only.

Introduction

Uric acid is the end product of purine metabolism and excreted in urine. High level of uric acid may cause solid crystals to form within joints. This cause a painful condition called gout. However high levels of uric acid may also be observed in kidney stones, cardiovascular disease, diabetes, alcoholisms, decreased renal functions etc. and low levels of uric acid is observed in multiple sclerosis and oxidative stress.

Method

Uricase-Peroxidase method - End Point.

Principle

Uric Acid is converted by uricase into allantoin and hydrogen peroxide. The hydrogen peroxide oxidizes the reaction product of 4- aminoantipyrine (4-AAP) with 3, 5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) in presence of a peroxidase to form a red colored dye complex.



Absorbance of the colored solution is directly proportional to the uric acid concentration, when measured at 505nm.

Reagent Composition

Reagent 1:

Peroxidase	>100U/L
Uricase	>100U/L
Ascorbate Oxidase	>100U/L
4-Amino Antipyrine	0.5 mmol/l

Reagent 2:

Uric acid Standard	6 mg/dl
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Precautions

Following precautions should be taken:

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- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.

Reagent Storage and Stability

Unopened reagents are stable till expiry mentioned on the label when stored at 2–8°C temperature.

Standard Reagent 2 is stable till expiry mentioned on the label when stored at 2-8°C.

Note: On request, Reagent 3(Uric acid:12 mg/dl) & Reagent 4 (Uric acid: 20 mg/dl) can be provided for linearity check with Reagent 2 (Uric acid: 6 mg/dl-Standard).

Reagent Preparation

Ready for use.

Reagent Deterioration

Reagents should be clear. Turbidity and/or precipitation may be because of reagent deterioration.

Sample Collection and Storage

Unhaemolysed serum or plasma can be used for the testing. Anti-coagulants like EDTA and heparin can be used. Do not use ammonium salts and sodium fluoride as anti-coagulants. It is recommended to use freshly collected samples for assay. Serum samples can be stored for 3 days at 2-8°C.

General Assay Parameters

Mode	End Point
Wavelength (nm)	505
Wavelength Range Usable (nm)	500-550
Blank with	Reagent
Sample Volume (µl)	25
Reagent R1 (µl)	1000
Incubation Time(min.)	10/5
Incubation Temperature(°C)	RT/37
Normal Low (mg/dl)	3.4
Normal High (mg/dl)	7
Linearity (mg/dl)	Upto 20
Standard Conc. (mg/dl)	6
Units	mg/dl

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Procedure

One reagent blank and one Standard are sufficient for each assay series.

Pipette into test tubes:

	Blank	Standard	Sample
Reagent 1	1000µl	1000µl	1000µl
Dist. Water	25µl	-	-
Reagent 2	-	25µl	-
Sample	-	-	25µl

Mix well & incubate for **5 min** at **37°C**. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 505nm.

Calculation

Uric Acid concentration in the sample can be calculated using the following formula:

$$\text{Uric Acid (mg/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

Example: If the absorbance of sample is 0.200 and the absorbance of standard is 0.18. The calculation shall be:

$$\frac{0.200}{0.180} \times 6 = 6.66 \text{ mg/dl}$$

If the uric acid concentration exceeds 20 mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

Male : 3.4 – 7.0 mg/dL

Female : 2.4 – 5.7 mg/dL

Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample: reagent, ratio remains same.
2. Hemolytic and lipemic samples may result in falsely elevated results. To avoid false results sample blank may be used as mentioned below:
 - Add 25µl of serum sample to 1000µl of DI water and read absorbance at 505nm.

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- Subtract the absorbance obtained as above, from the absorbance of test. Use this corrected absorbance for calculation.
- Reagents are sensitive to light and temperature. Reagents may develop a slight pink color on ageing. However, this does not interfere in the results.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for uric acid.

Performance

Linearity Limit: 20 mg/dl

Precision:

Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (mg/dl)	5.95	8.31
S.D.	0.12	0.13
C.V. %	2.00	1.59

Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (mg/dl)	5.86	8.31
S.D.	0.11	0.11
C.V. %	1.85	1.26

References

1. Aringer M, Graessler J (2008). "Understanding deficient elimination of uric acid". *Lancet* 372 (9654): 1929–1930. doi: 10.1016/S0140-6736(08)61344-6.
2. Becker BF (June 1993). "Towards the physiological function of uric acid". *Free radical biology & medicine* 14 (6): 615–31 doi: 10.1016/0891-5849(93)90143-1. PMID8325534 [http://linkinghub.elsevier.com/retrieve/pii/089-5849\(93\)90143-1](http://linkinghub.elsevier.com/retrieve/pii/089-5849(93)90143-1).

3. Chercey CC, Berger BJ, eds. (2004). *Laboratory Tests and Diagnostic Procedures*, 4th ed. Philadelphia: Saunders. <http://health.yahoo.com/arthritis-diagnosis/uric-acid-in-blood/health-wise--aa12023.html>
4. Wachtel M et al, Creation and Verification of Reference Intervals. *Laboratory Medicine* 1995; 26:593-7+uric acid www.thermo.com/eThermo/CMA/PDFs/Various/File_28398.pdf

Pack Presentation

Product Code/ Catalogue No.	Pack Size*	Reagent 1	Reagent 2
KGURA101.5.1	5x20 ml	5x20ml	1x2ml
KGURA101.5.2	5x50 ml	5x50ml	1x3ml











*Pack size may vary on market demand.

Revision No: (Ver: KGURA101.5/1)

Date of Issue: 1s April 2010

Symbols

Following symbols are used in the labeling of KEE GAD kits:

	Catalogue No.		Batch No.
	CE Mark		Read instructions
	In Vitro Diagnostics		Storage temperature
	Expiry Date		Content
	Product Name		Manufactured By



Manufactured by:

KEE GAD Biogen Pvt. Ltd.

**A-8, Third Floor, Naraina Industrial Area,
Phase-II, New Delhi-110028, (India)**