ANA BIO CLR URIC ACID

(Uricase-POD Method)

Intended Use

Uric acid is a reagent kit used for the determination of Uric acid based on enzymatic reactions using Uricase and Peroxidase enzymes.

Principle

Uricase converts Uric acid into allantoin and hydrogen peroxide (H_2O_2). In presence of Peroxidase, hydrogen peroxide oxidatively couples with phenolic chromogens to form a red coloured compound, which has maximum absorbance at 510 nm (500 - 530 nm). The concentration of the red coloured compound is proportional to the amount of uric acid in specimen.

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

$$\text{Phenolic chromogens} + 2\,H_2O_2 \xrightarrow{\text{POD}} \text{Red colour compound} + 3\,H_2O$$

Reagents provided

1. Uric acid reagent - Ready to use liquid reagent
2. Standard - Uric acid (6 mg/dl)

Reagent storage, stability and handling

The kit should be stored at 2° - 8℃ and is stable till the expiry date indicated on the label. DO NOT FREEZE THE REAGNET. The reagent should be stored only in the amber bottle provided to protect it from direct light. Before use swirl the reagents gently. DO NOT SHAKE VIGOROUSLY. Over the period of storage, the reagent may develop a light pink colour. This is expected and does not affect the reagent performance. Discard the reagent if the absorbance of the same exceeds 0.300 against distilled water blank at 510 nm. Contamination of the reagent should be strictly avoided. If the reagent develops turbidity discard the reagent.

Specimen collection and preservation

Blood should be collected in a clean and dry container. Avoid the use of plastic or siliconized container which may prolong clotting time. Serum or plasma should be separated from the cells at the earliest possible (with in 30 minutes). For plasma collection, following anticoagulants are used.

- EDTA 2 mg/ml of blood
- CITRATE 6 mg/ml of blood
- HEPARIN 200 IU/ml of blood
- OXALATE 3 mg/ml of blood
- SODIUM FLUORIDE 10 mg/ml of blood

In neatly separated serum/plasma Uric acid is stable for 3 days at room temperature (below 25℃) and for 6 months when stored at -20℃.

Assay guidelines for Analyzers

Reaction Type	End Point with Standard
Reaction slope	Increasing
Incubation time	5 min. at 37℃ / 10 min. at RT (25° - 30℃)
Wave length	510 nm (500 - 530nm)
Blank	Reagent Blank
Blank absorbance limit	< 0. 300 Abs against distilled water blank
Sample Volume	25 µl (0.025 ml)
Reagent Volume	1000 µl (1.0 ml)
Standard Con.	6 mg/ dl
Factor Calculation	6 mg/dl ÷ Absorbance of Standard.
Low normal	2.4 mg/dl
High normal	7.0 mg/dl
Linearity	Up to 25 mg/dl

Manual procedure

Bring the reagent and standard to room temperature before performing the assay.

Reagents	Blank	Standard	Sample
Reagent	1000 µl (1.0 ml)	1000 µl (1.0 ml)	1000 µl (1.0 ml)
Standard	-	25 µl (0.025 ml)	-
Sample	-	-	25 µl (0.025 ml)

1. Mix thoroughly and incubate at 37℃ for 5 minutes or 10 minutes at room temperature (25° - 30℃).
2. Read the absorbance against reagent blank at 510 nm (500 - 530nm).
3. The final colour is stable for 30 minutes if not exposed to direct light.

Calculation

Uric acid con. in sample (mg/dl) = $\frac{\text{Sample OD.}}{\text{Std. OD.}}$ x Con. of Std.

Normal Range

Male : 3.4 - 7.0 mg/dl
Female : 2.4 - 5.7 mg/dl

Note: Expected range varies from population to population and each laboratory should establish its own normal range.

Limitation

- The reagent is considered unsatisfactory and should not be used if its absorbance exceeds 0.300 at 510 nm against distilled water blank.
- The reaction is linear up to 25 mg / dl. For higher value, dilute sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.

Quality Control

To ensure adequate quality control measures, it is recommended that each batch should include a normal and an abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

References

1. Thefeld Wetal. Dtsch. Med Wechr., 98, 380 (1973).
2. Fossali P. et al, Clin Chem. 26, 227 (1980).

Symbols

IVD In Vitro Diagnostics. Caution. Keep away from sun light. Date of Manufacture.

LOT Batch No. Read Instructions. Fragile. Product Expiry Date.

CONT Content. Storage Temperature. Keep Dry. Manufactured By.

REF Catalogue No.

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