Uric Acid Reagent

Order No. Description
R80040 10 x 20 mL, with standard
R80041 10 x 50 mL, with standard

INTENDED USE
For the quantitative in vitro enzymatic determination of uric acid in serum or plasma at 520 nm.

TEST SUMMARY
Uric acid in the sample is oxidized by uricase to allantoin. In this reaction 1 mole of hydrogen peroxide is formed for every mole of uric acid oxidized according to the following equation:

\[
\text{Uric Acid + } \text{O}_2 + 2\text{H}_2\text{O} \rightarrow \text{Allantoin + CO}_2 + \text{H}_2\text{O}_2
\]

Hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene sulfonate (DHBS) and 4-aminooantipyrine (4-AAP) in a reaction catalyzed by horseradish peroxidase (HPOD), to give a quinoneimine dye. The intensity of the color of the solution of this dye is proportional to the concentration of uric acid in the sample:

\[
\text{HPOD}
\]

\[
2\text{H}_2\text{O}_2 + \text{DHBS} + 4-\text{AAP} \rightarrow \text{Quinoneimine dye + 4H}_2\text{O}
\]

The assay is carried out at 520 nm. The reaction is initiated by the addition of the sample to the reagent.

REAGENT COMPOSITION
Reactive ingredients:
- 4-Aminooantipyrine: 0.4 mmol/L
- 3,5-dichloro-2-hydroxybenzene sulfonate: 2 mmol/L
- Peroxidase: 5000 U/L
- Uricase: 50 U/L

Non-reactive ingredients:
- Buffers, stabilizers and fillers

REAGENT PREPARATION
Dissolve the dry powder in the vials with the volume of distilled or deionized water specified on the vial labels.

REAGENT STORAGE AND STABILITY
The unconstituted reagent is stable in the refrigerator (2–8 °C) until the expiration date printed on the label.

The uric acid reagent is stable after reconstitution in the refrigerator (2–8 °C) for at least 30 days and at room temperature (22–28 °C) for at least 2 days. If the freshly reconstituted reagent has an absorbance at 520 nm over 0.300, discard the reagent. A light pink coloration in a stored reagent is however normal. Another indication of reagent deterioration is when the reagent fails to recover stated values in control sera. If the reagent develops turbidity this indicates contamination. Do not use.

PRECAUTIONS
Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION AND STORAGE
Do not use hemolyzed samples. In addition to serum, EDTA or heparinized plasma can be used as samples.

Uric acid is reported (1) to be stable in serum for about 3 days in the refrigerator and for 6 months in frozen samples.

INTERFERING SUBSTANCES
A list of drugs affecting uric acid levels or interfering with its determination has been reported by Young et al. (2). Various substances and their lowest limits of interference in the assay using this same type of Trinder (3) reaction have been reported by Fossati et al. (4). These same authors report on the falsely low uric acid results obtained in the presence of ascorbic acid in the sample. They indicate however that ascorbic acid has disappeared from serum samples, as an interfering substance, in less than 90 minutes.

Bilirubin has been found to interfere in the determination of hydrogen peroxide in systems employing peroxidase, such as glucose, cholesterol and uric acid (5). This interference is minimized by the small amount of sample used, and by performing the assay at 520 nm, at which wavelength the absorbance of the sample due to turbidity and bilirubin is low.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Spectrophotometer or colorimeter capable of measuring absorbance accurately at 520 nm.
3. A constant temperature is not critical, but the assay should be carried out at the same temperature for the standard, the samples and control sera.
4. Distilled or deionized water.
5. Pipettes to measure distilled or deionized water, reagent, standard and samples.

MATERIALS PROVIDED
1. Uric acid reagent, in dry powder form.
2. Uric acid standard. Value of standard reported on label.

TEST PROCEDURE

<table>
<thead>
<tr>
<th>Material</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>1 mL reagent + 25 µL sample.</td>
</tr>
<tr>
<td>Standard</td>
<td>1 mL reagent + 25 µL sample.</td>
</tr>
<tr>
<td>Blank</td>
<td>1 mL reagent.</td>
</tr>
<tr>
<td>Incubate</td>
<td>10 minutes at 30 °C and 37 °C or 15 minutes at 25 °C.</td>
</tr>
<tr>
<td>Reading</td>
<td>Set instrument to 0 absorbance with the blank. Read absorbance of the test and standard.</td>
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</tbody>
</table>

CALIBRATION
Use the uric acid standard provided with this reagent. This standard may be diluted with water to produce a lower concentration; the standard should be diluted as needed and not stored.

QUALITY CONTROL
Sera samples are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established concentrations for uric acid may be used. Assayed Control Serum Level 1 (Cat. No. R83082) and Level 2 (Cat. No. R83083) are recommended for this purpose.

CALCULATIONS

\[
C_s = \text{Value of the standard in mg/dL Uric acid.}
\]

\[
C_a = \frac{C_s \times V_s}{V_a} = \text{Uric acid in sample in mg/dL.}
\]

A standard

LIMITATIONS OF THE PROCEDURE
1. Samples with uric acid concentrations exceeding the linearity of this assay (20 mg/dL) should be diluted with an equal volume of physiological saline (0.85% NaCl w/v in water) and assayed again; multiply results by 2.
2. Incubation times for the reaction to reach completion may be longer than indicated in "TEST PROCEDURE" section, particularly with reagents reconstituted and stored for a prolonged time. Insure that the reaction has reached completion at the selected temperature.
3. Even though the absorbance of the sample at 520 nm is minimal, a blank should be run for extremely turbid sera, by adding 25 µL of sample into 1 mL of 0.150M potassium phosphate buffer pH 7.5. The absorbance of this blank should be subtracted from that of the respective assay.
4. When using spectrophotometers with cuvettes requiring a larger sample, provide additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)
REAGENT PERFORMANCE
1. Linearity: The assay is linear to 20 mg/dL.
2. Correlation: Employing as a reference the Uricase/Catalase method of Haeckel (6) in 60 serum samples ranging in concentration from 2.9 to 12.6 mg/dL, the correlation coefficient was 0.994 and the regression equation was
   \[ y = 0.980 \times -0.131. \]
3. Precision:

   Within Run
   \[
   \begin{array}{l|c|c|c}
   \text{Mean (mg/dL)} & 4.81 & 8.36 & 13.69 \\
   \text{SD} & 0.043 & 0.081 & 0.1 \\
   \text{CV} & 0.89 & 0.97 & 0.73 \\
   \text{N} & 12 & 12 & 12 \\
   \end{array}
   \]

   Run to Run
   \[
   \begin{array}{l|c|c|c}
   \text{Mean (mg/dL)} & 4.85 & 8.35 & 13.65 \\
   \text{SD} & 0.073 & 0.076 & 0.137 \\
   \text{CV} & 1.5 & 0.91 & 1 \\
   \text{N} & 35 & 35 & 33 \\
   \end{array}
   \]

REFERENCE RANGE (1)
Men 3.6-7.7 mg/dL.
Women 2.5-6.8 mg/dL.
It is recommended that every laboratory establish its own reference range.

REFERENCES

RE-ORDER INFORMATION
Uric Acid Reagent
Catalog No. R80040
Catalog No. R80041
Made in the USA

For in vitro diagnostic use

See package insert for proper use

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