Uric Acid Assay Kit

Item No. 700320

www.caymanchem.com
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GENERAL INFORMATION

Materials Supplied

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>Quantity/Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>700321</td>
<td>Uric Acid Assay Buffer (10X)</td>
<td>1 vial/5 ml</td>
<td>-20°C</td>
</tr>
<tr>
<td>700322</td>
<td>Uric Acid Fluorometric Detector</td>
<td>2 vials</td>
<td>-20°C</td>
</tr>
<tr>
<td>700323</td>
<td>Uric Acid Enzyme Mixture</td>
<td>2 vials</td>
<td>-20°C</td>
</tr>
<tr>
<td>700001</td>
<td>DMSO Assay Reagent</td>
<td>1 vial/1 ml</td>
<td>-20°C</td>
</tr>
<tr>
<td>700325</td>
<td>Uric Acid Standard</td>
<td>2 vials</td>
<td>-20°C</td>
</tr>
<tr>
<td>400017</td>
<td>96-Well Plate (black)</td>
<td>1 plate</td>
<td>RT</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>1 cover</td>
<td>RT</td>
</tr>
</tbody>
</table>

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.
Safety Data
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

Precautions
Please read these instructions carefully before beginning this assay.

If You Have Problems
Technical Service Contact Information
Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability
This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied
1. A fluorometer with the capacity to measure fluorescence using an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background
Uric acid (urate) is the end product of human purine metabolism and is mainly excreted in urine. Serum uric acid concentrations are dependent upon the rate of uric acid produced (via purine metabolism) and renal uric acid excretion. Most animals can metabolize uric acid to the more readily excreted product, allantoin. However, humans lack the necessary enzyme for conversion of uric acid to allantion, urate oxidase (uricase), due to the presence of two nonsense mutations in the human uricase gene.¹ Many factors, including genetic components and acquired factors, such as obesity and alcohol consumption, influence serum uric acid concentrations.² Hyperuricemia induces or facilitates gout, kidney stones, metabolic syndrome, hypertension, and renal and cardiovascular disease, while exercise-induced acute renal failure is a significant complication of renal hypouricemia.³-⁶
About This Assay

Cayman's Uric Acid Assay provides a fluorescence-based method for detecting uric acid in serum, plasma, and urine. In the assay, uricase catalyzes the conversion of uric acid to allantoin, hydrogen peroxide ($H_2O_2$), and carbon dioxide. $H_2O_2$, in the presence of horseradish peroxidase, reacts stoichiometrically with ADHP (10-acetyl-3,7-dihydroxyphenoxazine) to produce the highly fluorescent compound resorufin (Figure 1). Resorufin fluorescence can be analyzed with an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm.

![Figure 1. Assay scheme](image)

**Uric acid + $O_2 + 2H_2O \xrightarrow{Uricase} Allantoin + CO_2 + H_2O_2**

$H_2O_2 + ADHP \xrightarrow{Horseradish Peroxidase} Resorufin + 2H_2O$

$\text{Ex}_{530}/\text{Em}_{590}$

**PRE-ASSAY PREPARATION**

**Reagent Preparation**

1. **Uric Acid Assay Buffer (10X) - (Item No. 700321)**
   The vial contains 5 ml of 1 M Tris-HCl (pH 7.5). Dilute 3 ml of Assay Buffer concentrate with 27 ml of HPLC-grade water. This final 1X Assay Buffer (100 mM Tris-HCl, pH 7.5) is used in the assay. The 1X buffer is stable for six months at 4°C.

2. **Uric Acid Enzyme Mixture - (Item No. 700323)**
   The vials contain a lyophilized powder of uricase and horseradish peroxidase. Reconstitute the contents of the vial with 900 µl of 1X Assay Buffer and place on ice. This is enough enzyme mixture to assay 60 wells. Prepare the additional vial as needed. The reconstituted enzymes are stable for four hours on ice.

3. **DMSO Assay Reagent - (Item No. 700001)**
   The vial contains 1 ml of dimethylsulfoxide (DMSO). The reagent is ready to use as supplied. Once thawed, DMSO may be stored at room temperature. It is stable at room temperature for six months.

4. **Uric Acid Standard - (Item No. 700325)**
   The vials contain a lyophilized powder of uric acid. Reconstitute the contents of the vial with 1 ml of 1X Assay Buffer. The concentration of this uric acid stock solution is 0.5 mM. It is ready to use to prepare the standard curve. The reconstituted Standard is stable for four hours at room temperature.

5. **Uric Acid Fluorometric Detector - (Item No. 700322)**
   The vials contain a clear lyophilized powder of ADHP (10-acetyl-3,7-dihydroxyphenoxazine). Immediately prior to assaying, add 90 µl of DMSO (Item No. 700001) to the vial, vortex, and then add 810 µl of 1X Assay Buffer. This is enough detector to assay 60 wells. Prepare the additional vial as needed. The reconstituted mixture is stable for 60 minutes. After 60 minutes, increased background fluorescence will occur.
Sample Preparation

Plasma

Typically, normal human plasma has an uric acid concentration of ~200-500 µM.

1. Collect blood using an anticoagulant such as heparin, EDTA, or sodium citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice.
3. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.

Serum

Typically, normal human serum has an uric acid concentrations of ~200-400 µM.

1. Collect blood without using an anticoagulant.
2. Allow the blood to clot for 30 minutes at 25°C.
3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice.
4. If not assaying the same day, freeze at -80°C. The serum sample will be stable for one month while stored at -80°C.

Urine

Typically, normal human urine has an uric acid concentration of 1.5-4.5 mmol/24 hours or 250-750 mg/24 hours.

1. Collection of urine does not require any special treatments.
2. Dilute urine 1:5-1:50 with diluted Assay Buffer before assaying.
3. If not assaying the same day, dilute urine 1:5-1:10 with diluted Assay Buffer, and freeze at -80°C. The diluted urine sample will be stable for one month while stored at -80°C. It will be ready to assay once thawed; do not dilute further.

NOTE: Uric acid values from urine samples can be normalized to creatinine levels using Cayman’s Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701).
ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a uric acid standard curve in duplicate has to be assayed with the samples. We suggest that each sample be assayed at least in duplicate. A typical layout of standards and samples to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 18).

<table>
<thead>
<tr>
<th></th>
<th>1</th>
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<th>3</th>
<th>4</th>
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</tr>
</tbody>
</table>

S1-S8 - Standards A-H
1-40 - Samples

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 150 µl in all the wells.
- All reagents except the Enzyme Mixture must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying samples in triplicate, but it is the user’s discretion to do so.
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm.
Standard Preparation

Take eight clean glass test tubes and mark them A-H. Add the amount of uric acid stock (0.5 mM) and diluted Assay Buffer to each tube as described in Table 1. The diluted Standards are stable for four hours at room temperature.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Uric acid stock (μl)</th>
<th>Assay Buffer (μl)</th>
<th>Final Concentration (µM) in plate well</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>198</td>
<td>0.5</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>196</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>192</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>16</td>
<td>184</td>
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</tr>
<tr>
<td>F</td>
<td>24</td>
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</tr>
<tr>
<td>G</td>
<td>32</td>
<td>168</td>
<td>8</td>
</tr>
<tr>
<td>H</td>
<td>40</td>
<td>160</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 1. Preparation of uric acid standards

Performing the Assay

1. **Standard Wells** - add 105 µl of diluted Assay Buffer, 15 µl Fluorometric Detector, and 15 µl of Standard (tubes A-H) per well in the designated wells on the plate (see Sample plate format, Figure 2, page 10).
2. **Sample Wells** - add 105 µl of diluted Assay Buffer, 15 µl of Fluorometric Detector, and 15 µl of sample to at least two wells.
3. Initiate the reactions by adding 15 µl of Enzyme Mixture to all of the wells being used.
4. Incubate the plate for fifteen minutes at room temperature and then read using an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm. Fluorescence is stable up to 30 minutes.
Calculations

1. Determine the average fluorescence of each standard and sample.
2. Subtract the fluorescence value of standard A from itself and all other standards and samples. This is the corrected fluorescence (CF).
3. Plot the corrected fluorescence values (from step 2 above) of each standard as a function of the final concentration of uric acid from Table 1. See Figure 3, on page 15, for a typical standard curve.
4. Calculate the uric acid concentration of the samples using the equation below.

\[
\text{Uric acid (µM)} = \left( \frac{\text{CF} - (\text{y-intercept})}{\text{Slope}} \right) \times \frac{0.15 \text{ ml}}{0.015 \text{ ml}} \times \text{Sample dilution}
\]

NOTE: Uric acid values from urine samples can be normalized to creatinine levels using Cayman's Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701).

Performance Characteristics

Precision:
When a series of 16 urine and 16 serum measurements were performed on the same day, the intra-assay coefficient of variation was 1.8% and 4.1%, respectively. When a series of 16 urine and 16 serum measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 2.2% and 4.3%, respectively.

Assay Range:
Under the standardized conditions of the assay described in the booklet, the dynamic range of the assay is 0.5-10 µM of uric acid.
## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic values; dispersion of duplicates/triplicates</td>
<td>A. Poor pipetting/technique</td>
<td>A. Be careful not to splash the contents of the wells</td>
</tr>
<tr>
<td></td>
<td>B. Bubble in the well(s)</td>
<td>B. Carefully tap the side of the plate with your finger to remove bubbles</td>
</tr>
<tr>
<td>No fluorescence was detected above background in sample wells</td>
<td>Sample was too dilute</td>
<td>Re-assay the sample using a lower dilution</td>
</tr>
<tr>
<td>Fluorometer exhibited ‘MAX’ values for the wells</td>
<td>The GAIN setting is too high</td>
<td>Reduce the GAIN and re-read</td>
</tr>
<tr>
<td>Fluorescence in the sample wells are above the last standard</td>
<td>Sample is too concentrated</td>
<td>Re-assay the sample using a higher dilution</td>
</tr>
</tbody>
</table>

### References

NOTES

Warranty and Limitation of Remedy
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