



Uric Acid Assay Kit

Item No. 700320

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
700321	Uric Acid Assay Buffer (10X)	1 vial/5 ml	-20°C
700322	Uric Acid Detector	2 vials	-20°C
700323	Uric Acid Enzyme Mixture	2 vials	-20°C
700001	DMSO Assay Reagent	1 vial/1 ml	RT
700325	Uric Acid Standard	2 vials	-20°C
400017	96-Well Plate (black)	1 plate	RT
400014	96-Well Solid Plate	1 plate	RT
400012	96-Well Cover Sheet	1 ea	RT

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

Email: techserv@caymanchem.com

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 as directed in the **Materials Supplied** section (see page 3) 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 or a plate reader capable of measuring absorbance at 570 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass-distilled or HPLC-grade water is acceptable

Background

Uric acid (urate) is the end-product of human purine metabolism.¹ In most organisms, uric acid is metabolized to allantoin by the enzyme uricase, which results in low plasma uric acid levels. However, humans and apes have mutations in the uricase gene that result in a non-functional enzyme, leading to higher plasma levels of uric acid than those found in other mammals.² The levels of circulating uric acid in humans are also increased in hypoxanthine-guanine phosphoribosyl transferase (HGPRT) deficiency, phosphoribosyl pyrophosphate synthetase (PRPP) overactivity, or by lifestyle factors, such as alcohol consumption, among others.¹ Hyperuricemia is positively correlated with the incidence of gout and is also associated with kidney stones, metabolic syndrome, hypertension, and cardiovascular disease.³⁻⁵ Reduced levels of uric acid are present in disorders of decreased urate production or increased excretion, such as xanthinuria or renal hypouricemia, respectively.⁶ Renal hypouricemia is associated with mutations in urate transporter 1 (URAT1) or glucose transporter 9 (GLUT9) and with the risk of exercise-induced acute kidney injury.

About This Assay

Cayman's Uric Acid Assay Kit provides fluorescence- or colorimetric-based methods for detecting uric acid in plasma, serum, and urine. In the assay, uricase catalyzes the conversion of uric acid to allantoin, hydrogen peroxide (H₂O₂), and carbon dioxide. H₂O₂, 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. Alternatively, the absorbance of resorufin can be measured at 570 nm.

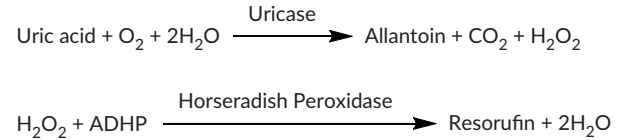


Figure 1. Assay scheme

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 Uric Acid Assay Buffer (10X) with 27 ml of pure water. This final Uric Acid Assay Buffer (1X) (100 mM Tris-HCl, pH 7.5) is used in the assay. The Uric Acid Assay Buffer (1X) will be 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 Uric Acid Assay Buffer (1X) and place on ice. This is enough Uric Acid Enzyme Mixture to assay 60 wells. Prepare the additional vial as needed. The reconstituted Uric Acid Enzyme Mixture will be stable for four hours on ice.

3. DMSO Assay Reagent - (Item No. 700001)

The vial contains 1 ml of DMSO. The reagent is ready to use as supplied. Once thawed, DMSO may be stored at room temperature. It will be 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 0.5 ml of Uric Acid Assay Buffer (1X). The concentration of this uric acid stock solution is 1 mM. It is ready to use to prepare the standard curve. The reconstituted Uric Acid Standard will be stable for four hours at room temperature.

5. Uric Acid Detector - (Item No. 700322)

The vials contain a clear, lyophilized powder of ADHP. Immediately prior to assaying, add 90 µl of DMSO to the vial, vortex, and then add 810 µl of Uric Acid Assay Buffer (1X). This is enough Uric Acid Detector to assay 60 wells. Prepare the additional vial as needed. The reconstituted mixture will be stable for 60 minutes. After 60 minutes, increased background fluorescence or absorbance will occur.

Sample Preparation

Plasma

Typically, normal human plasma has a uric acid concentration less than 7 mg/dl (420 µM).⁷

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.
4. Dilute plasma 1:2-1:25 with Uric Acid Assay Buffer (1X) before assaying.

Serum

Typically, normal human serum has a mean uric acid concentration of 4-5 mg/dl (240-300 μM).⁷

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.
5. Dilute serum 1:2-1:25 with Uric Acid Assay Buffer (1X) before assaying.

Urine

Typically, normal human urine has a uric acid concentration of 619-684 mg/24 hours (3.7-4.1 mmol/24 hours) in males and 532-590 mg/24 hours (3.2-3.5 mmol/24 hours) in females.⁸

1. Collection of urine does not require any special treatments.
2. If not assaying the same day, freeze at -80°C, where it will be stable for one month.
3. Dilute urine 1:5-1:50 with Uric Acid Assay Buffer (1X) before assaying.

It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine ELISA Kit (Item No. 502330), Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a uric acid standard curve in duplicate must 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 21).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	9	9	17	17	25	25	33	33
B	S2	S2	2	2	10	10	18	18	26	26	34	34
C	S3	S3	3	3	11	11	19	19	27	27	35	35
D	S4	S4	4	4	12	12	20	20	28	28	36	36
E	S5	S5	5	5	13	13	21	21	29	29	37	37
F	S6	S6	6	6	14	14	22	22	30	30	38	38
G	S7	S7	7	7	15	15	23	23	31	31	39	39
H	S8	S8	8	8	16	16	24	24	32	32	40	40

S1-S8 - Standard A-H wells
1-40 - Sample wells

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 Uric Acid 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 at 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; monitor the absorbance at 570 nm. *NOTE: This assay can be read using fluorescence or absorbance. You do not need to prepare both standard curves; choose the one that matches the format you will be using.*

Standard Curve Preparation - Fluorometric

Take eight clean test tubes and mark them A-H. Add the amount of uric acid stock solution (1 mM) and Uric Acid Assay Buffer (1X) to each tube as described in Table 1. The diluted standards will be stable for four hours at room temperature.

Tube	Uric Acid (μl)	Uric Acid Assay Buffer (1X) (μl)	Final Concentration (μM)
A	0	400	0
B	2	398	5
C	4	396	10
D	8	392	20
E	16	384	40
F	24	376	60
G	32	368	80
H	40	360	100

Table 1. Preparation of the uric acid standards - fluorometric

Standard Curve Preparation - Colorimetric

Take eight clean test tubes and mark them A-H. Add the amount of uric acid stock solution (1 mM) and Uric Acid Assay Buffer (1X) to each tube as described in Table 2. The diluted standards will be stable for four hours at room temperature.

Tube	Uric Acid (μ l)	Uric Acid Assay Buffer (1X) (μ l)	Final Concentration (μ M)
A	0	100	0
B	5	95	50
C	10	90	100
D	20	80	200
E	40	60	400
F	60	40	600
G	80	20	800
H	100	0	1,000

Table 2. Preparation of the uric acid standards - colorimetric

Performing the Assay

NOTE: Use the 96-Well Plate (Black) (Item No. 400017) for the fluorometric assay and the 96-Well Solid Plate (Item No. 400014) for the colorimetric assay.

- Standard Wells** - add 105 μ l of Uric Acid Assay Buffer (1X), 15 μ l Uric Acid Detector, and 15 μ l of standard (tubes A-H) per well in the designated wells on the plate (see **Sample plate format**, Figure 2, page 11).
- Sample Wells** - add 105 μ l of Uric Acid Assay Buffer (1X), 15 μ l of Uric Acid Detector, and 15 μ l of sample to at least two wells.
- Initiate the reactions by adding 15 μ l of Uric Acid Enzyme Mixture to all of the wells being used.
- Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for fifteen minutes at room temperature. Remove the cover and read using an excitation wavelength between 530-540 nm and an emission wavelength between 585-595 nm. Fluorescence will be stable up to 30 minutes. If the colorimetric method is used, read absorbance at 570 nm.

Calculations

1. Determine the average fluorescence or absorbance of each standard and sample.
2. Subtract the fluorescence or absorbance value of standard A from itself and all other standards and samples. This is the corrected signal (CS).
3. Plot the CS values (from step 2 above) of each standard as a function of the final concentration of uric acid from Table 1 on page 13 or Table 2 on page 14, respectively. See Figures 3 and 4, on pages 17 and 18, for typical standard curves. For ease of calculation, the plot could be constructed with concentration on the y-axis and the CS on the x-axis.
4. Calculate the uric acid concentration of the samples using the second-order polynomial equation below.

$$\text{Uric acid } (\mu\text{M}) = \left[A \times (\text{CS})^2 + B \times (\text{CS}) + C \right] \times \text{Sample dilution}$$

Performance Characteristics

Sensitivity:

The lower limit of quantification (LLOQ) for the fluorometric assay is 5 μM and the lower limit of detection (LLOD) is 0.18 μM . The LLOQ for the colorimetric assay is 50 μM and the LLOD is 0.45 μM .

Precision:

When a series of 16 urine and 16 serum measurements were performed on the same day, the intra-assay coefficients of variation were 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 coefficients of variation were 2.2 and 4.3%, respectively.

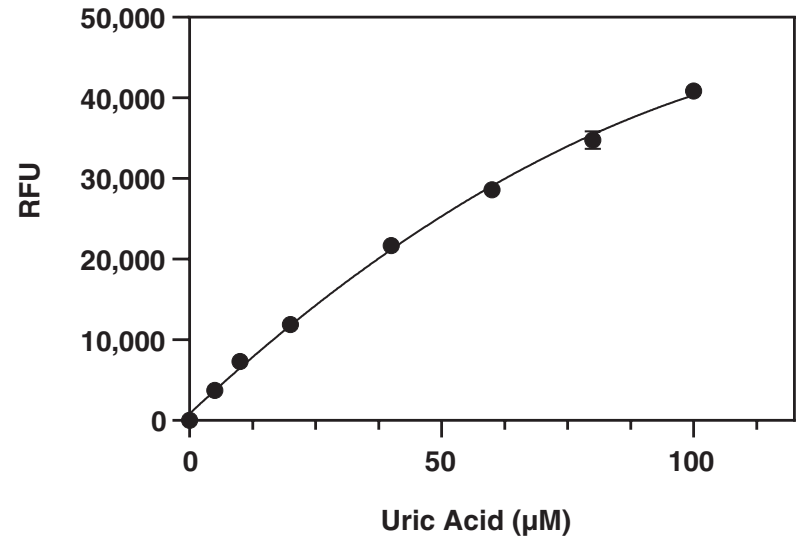


Figure 3. Uric acid standard curve - fluorometric

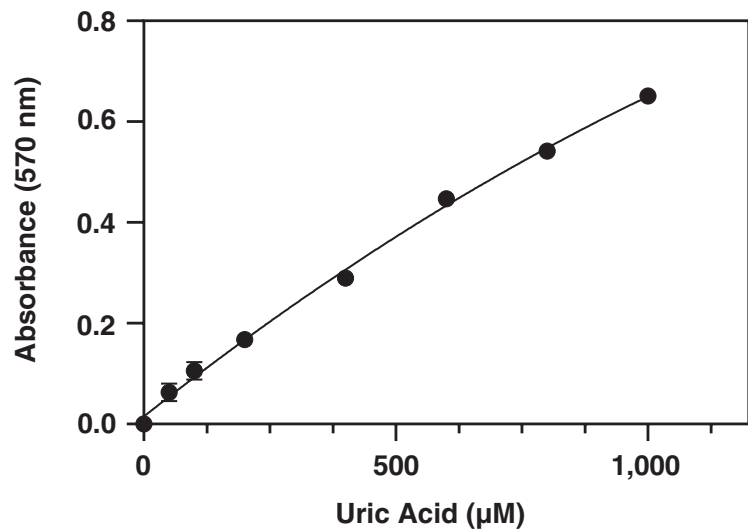


Figure 4. Uric acid standard curve - colorimetric

RESOURCES

Troubleshooting

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

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12								
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3								
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1								
	A	B	C	D	E	F	G	H

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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