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; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled or HPLC-grade water is acceptable
4. Materials used for Sample Preparation (see page 9)

INTRODUCTION

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 (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. Alternatively, the absorbance of resorufin can be measured at 570 nm.

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

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

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 one vial with 900 µl of Uric Acid Assay Buffer (1X) and place on ice. This is a sufficient volume of 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 and will be stable for six months.

4. **Uric Acid Standard - (Item No. 700325)**

   The vials contain a lyophilized powder of uric acid. Reconstitute the contents of one vial with 0.5 ml of Uric Acid Assay Buffer (1X). The concentration of this uric acid stock solution is 1 mM, which is the concentration needed 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, mix gently, then add 810 µl of Uric Acid Assay Buffer (1X). This a sufficient volume of 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

To obtain more consistent results, it is recommended to deproteinate all plasma and serum samples for the fluorometric assay (see the **Plasma/Serum Deproteination** section, on page 11). It is not necessary to deproteinate plasma and serum samples for the colorimetric assay unless the samples contain uricase or other substances that can interfere with the assay.

Plasma

Typically, normal human plasma has a uric acid concentration of 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 the plasma on ice.
3. If not assaying the same day, freeze at -80°C. The plasma samples will be stable for one month while stored at -80°C.

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 the serum on ice.
4. If not assaying the same day, freeze at -80°C. The serum samples will be stable for one month while stored at -80°C.

Plasma/Serum Deproteination

Materials Needed

1. Methanol

Deproteination Protocol

1. Pipette a known amount of sample into a clean tube.
2. Add 4X the sample volume of ice-cold methanol.
3. Vortex thoroughly and incubate on ice for 5 minutes.
4. Centrifuge the sample at 10,000 x g at 4°C for 10 minutes.
5. Transfer the supernatant into a clean glass tube.
6. Follow steps 3-5 two more times and combine the supernatants.
7. Evaporate the methanol to dryness under a stream of nitrogen (samples can be heated to 37°C to accelerate evaporation).
8. Resuspend the sample in Uric Acid Assay Buffer (1X) to its original volume. Samples can be further diluted with the same buffer, if necessary. Generally, human plasma and serum should be diluted 1:2-1:25 with Uric Acid Assay Buffer (1X) prior to the assay to be in range of the standard curves.

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 treatment.
2. If not assaying the same day, freeze at -80°C, where the urine samples 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.

NOTE: Do not use Cayman's Creatinine ELISA Kit (Item No. 502330) for mouse samples

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. It is suggested that each sample and standard be assayed at least in duplicate (triplicate is preferred). A typical layout of standards and samples to be measured in duplicate is shown below in Figure 2. It is suggested that the contents of each well are recorded on the template sheet provided (see page 30).

	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 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 in 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.
- It is recommended that the samples be assayed at least in duplicate (triplicate is preferred), 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

Label eight clean test tubes 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, below. 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

Label eight clean test tubes 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, below. 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	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.

1. **Standard Wells -** add 105 µl of Uric Acid Assay Buffer (1X), 15 µl of Uric Acid 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 13).
2. **Sample Wells -** add 105 µl of Uric Acid Assay Buffer (1X), 15 µl of Uric Acid Detector, and 15 µl of sample to at least two wells.
3. Initiate the reactions by adding 15 µl of Uric Acid Enzyme Mixture to all of the wells being used.
4. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for 15 minutes for the fluorometric assay or 30 minutes for the colorimetric assay at room temperature protected from light.
5. 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 for up to 30 minutes. If the colorimetric method is used, read absorbance at 570 nm.

ANALYSIS

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 16 for the fluorometric assay or Table 2 on page 17 for the colorimetric assay. See Figures 3 and 4, on pages 21 and 22, 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.

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

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, in the fluorometric assay. 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, in the fluorometric assay.

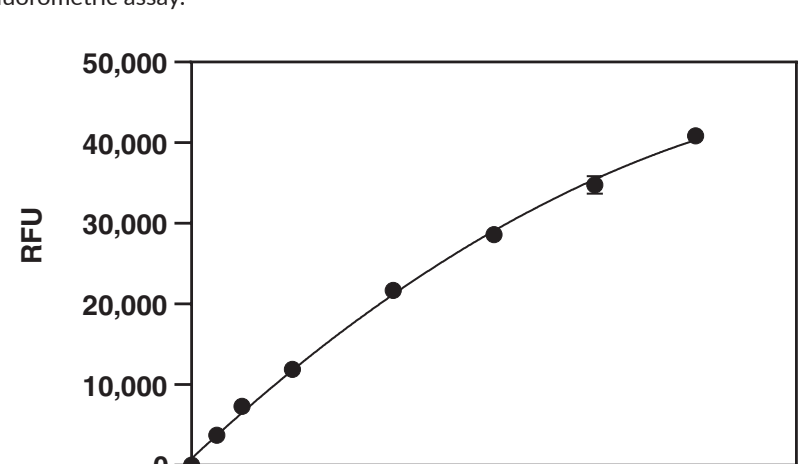


Figure 3. Uric acid standard curve - fluorometric

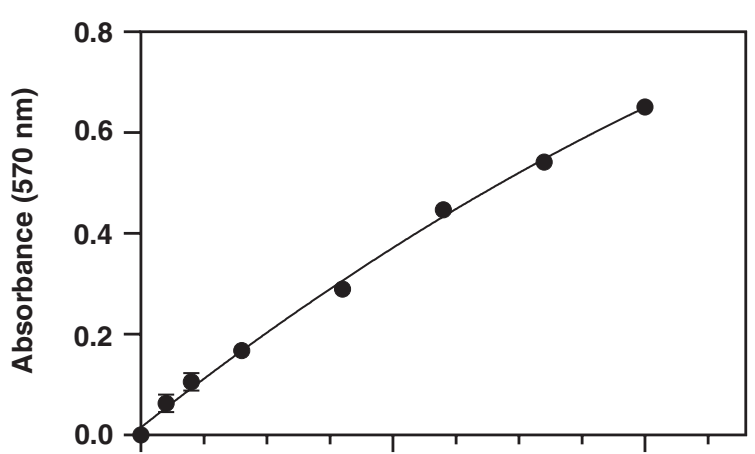


Figure 4. Uric acid standard curve - colorimetric

Spike and Recovery

Human and mouse samples were spiked with uric acid, deproteinized, serially diluted with Uric Acid Assay Buffer (1X), and evaluated using the Uric Acid Assay Kit (fluorometric). The error bars represent standard deviations obtained from multiple dilutions of each sample.

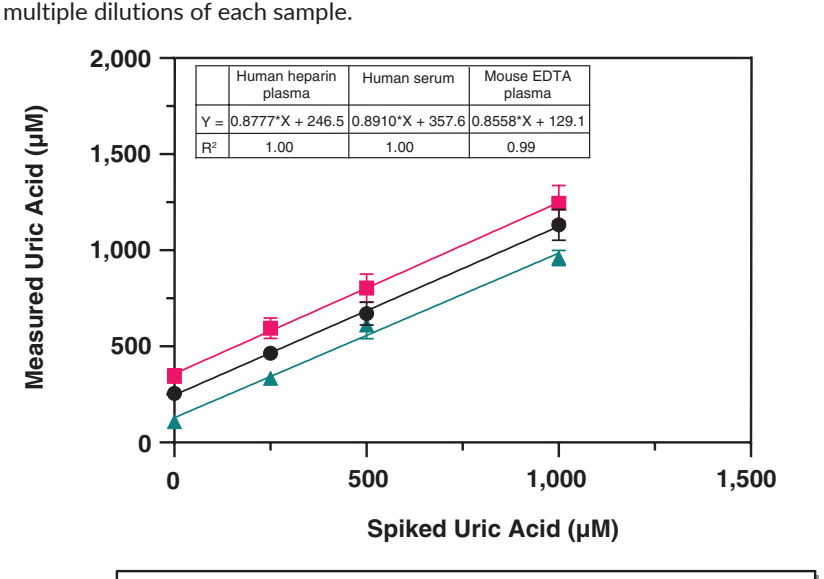


Figure 5. Spike and recovery of uric acid in various matrices - fluorometric

Parallelism

To assess parallelism in the fluorometric assay, samples were processed as described in the Sample Preparation section, serially diluted with Uric Acid Assay Buffer (1X), and evaluated using the Uric Acid Assay Kit (fluorometric). Measured concentrations were plotted as a function of sample dilution. The results are shown below.

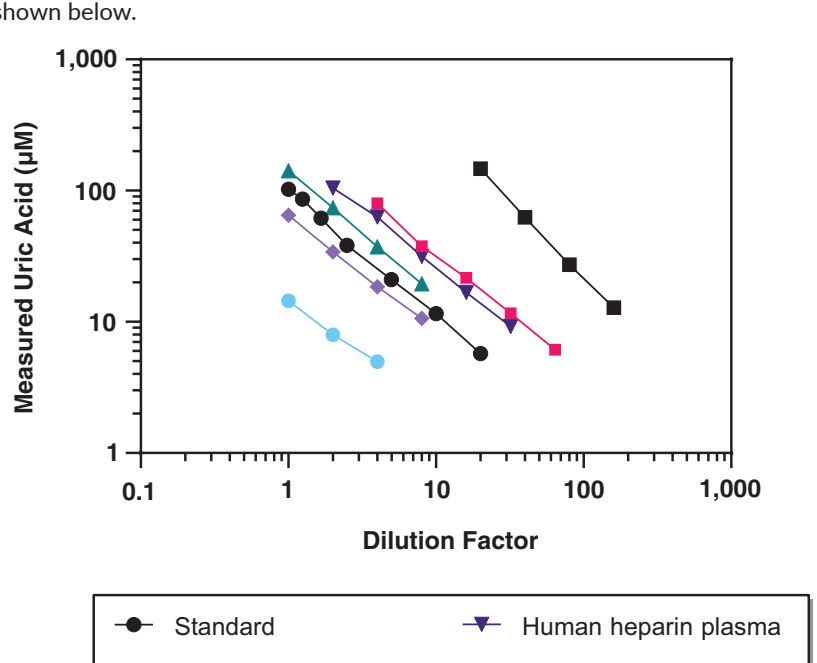


Figure 6. Parallelism of various matrices - fluorometric

To assess parallelism in the colorimetric assay, samples were processed as described in the Sample Preparation section, serially diluted with Uric Acid Assay Buffer (1X), and evaluated using the Uric Acid Assay Kit (colorimetric). Measured concentrations were plotted as a function of sample dilution. The results are shown below.

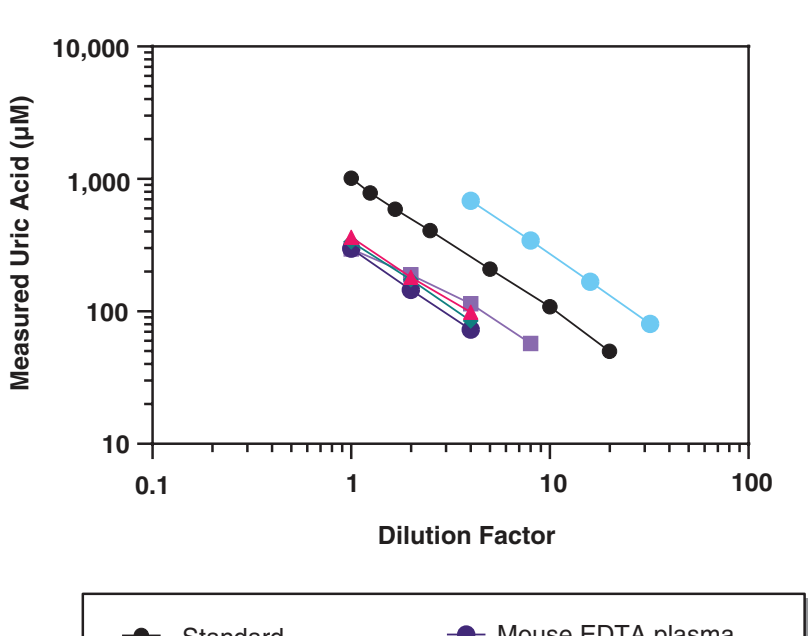


Figure 7. Parallelism of various matrices - colorimetric

Linearity

Human and mouse samples were spiked with uric acid, processed as described in the Sample Preparation section, serially diluted with Uric Acid Assay Buffer (1X), and evaluated for linearity using the Uric Acid Assay Kit. The results are shown in the table below and on page 27.

Dilution	Uric Acid (µM)	Linearity (%)
Human EDTA plasma, spiked with 2.5 mM uric acid		
24	2,780	100
48	2,428	87
96	2,554	92
192	2,636	95
Human serum, spiked with 1 mM uric acid		
16	1,091	100
32	1,230	113
64	1,309	120
128	1,279	117
256	1,314	120
Mouse EDTA plasma, spiked with 1 mM uric acid		
16	905	100
32	983	109
64	959	106
128	993	110

Table 3. Linearity in various matrices - fluorometric

Dilution	Uric Acid (µM)	Linearity (%)
Human EDTA plasma, spiked with 1 mM uric acid		
2	1,195	100
4	1,323	111
8	1,440	121
Human serum, spiked with 1 mM uric acid		
2	1,233	100
4	1,300	105
8	1,328	108
Mouse EDTA plasma, spiked with 1 mM uric acid		
2	1,134	100
4	1,159	102
8	1,167	103

Table 4. Linearity in various matrices - colorimetric

*NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted / First observed concentration value in the dilution series, dilution adjusted)*100*

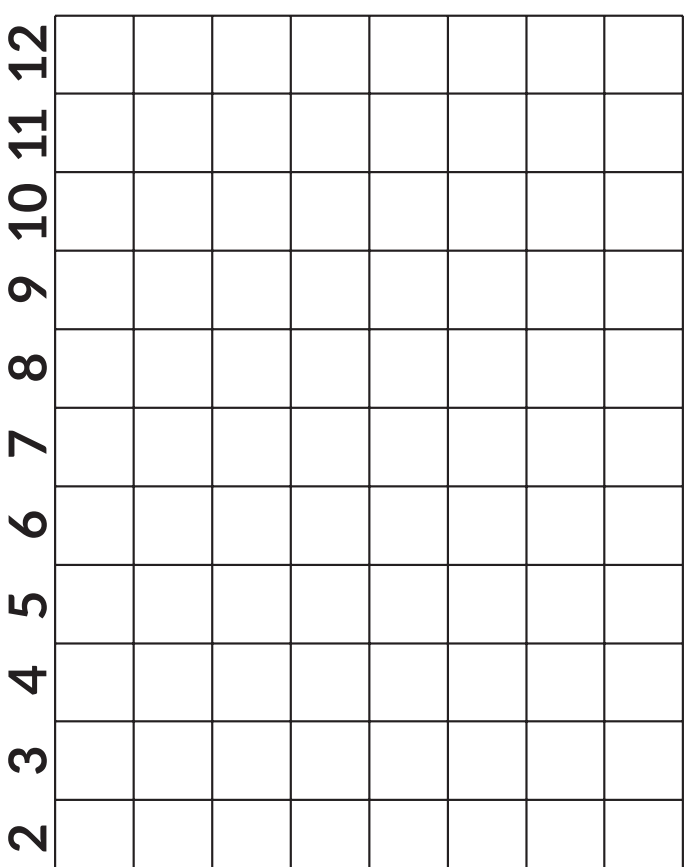
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	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 *gain* setting is too high	Reduce the *gain* 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

References

1. Ichida, K. What lies behind serum urate concentration? Insights from genetic and genomic studies. *Genome Med.* **1(12)**, 118 (2009).
2. Watanabe, S., Kang, D.-H., Feng, L., *et al.* Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. *Hypertension* **40(3)**, 355-360 (2002).
3. Nakagawa, T., Hu, H., Zharikov, S., *et al.* A causal role for uric acid in fructose-induced metabolic syndrome. *Am. J. Physiol. Renal Physiol.* **290(3)**, F625-F631 (2006).
4. Day, R.O., Graham, G.G., Hicks, M., *et al.* Clinical pharmacokinetics and pharmacodynamics of allopurinol and oxypurinol. *Clin. Pharmacokinet.* **46(8)**, 623-644 (2007).
5. Yu, W and Cheng, J.-D. Uric acid and cardiovascular disease: An update from molecular mechanism to clinical perspective. *Front. Pharmacol.* **11**, 582680 (2020).
6. Otani, N., Ouchi, M., Misawa, K., *et al.* Hypouricemia and urate transporters. *Biomedicines* **10(3)**, 652 (2022).
7. Baldree, L.A. and Stapleton, F.B. Uric acid metabolism in children. *Pediatr. Clin. North Am.* **37(2)**, 391-418 (1990).
8. Sila-On, A., Pavaro, U., and Nuchpramool, W. Serum and urinary uric acid levels in healthy subjects and in patients with urolithiasis. *J. Med. Assoc. Thai.* **74(8)**, 352-357 (1991).



NOTES

Warranty and Limitation of Remedy

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