Urea Fluorometric Assay Kit

Item No. 700620

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, store kit as supplied or remove components and store as stated below.

Item Number	Item	Quantity/ Size	Storage
700621	Potassium Phosphate Buffer (500 mM; pH 7.0)	1 vial/5 ml	RT
700622	Urease Assay Reagent	2 vials	-20°C
700623	Urea Standard	2 vials	-20°C
700624	Urea Ammonia Detector	2 vials	-20°C
700566	Ethanol Assay Reagent	1 vial/2 ml	RT
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	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, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the capacity to measure fluorescence using an excitation wavelength of 405-415 nm and an emission wavelength of 470-480 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

Urea or carbamide is synthesized in the body of many organisms as part of the urea cycle.[1] It is the final degradation product of protein and amino acid metabolism. In protein catabolism, proteins are broken down to amino acids and deaminated. The ammonia formed in this process is converted to urea in the liver and is regulated by N-acetylglutamate. Urea is found in blood and is excreted by the kidney as a component of urine.[1] Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons, allowing for re-absorption of water and critical ions from the excreted urine.[2,3]

The determination of urea is the most widely used test for the evaluation of kidney function. The test is frequently used in conjunction with the determination of creatinine for the differential diagnosis of prerenal hyperuremia (cardiac decompensation, water depletion, increased protein catabolism), renal hyperuremia (glomerulonephritis, chronic nephritis, polycystic kidney, nephrosclerosis, tubular necrosis), and post renal hyperuremia (obstructions of the urinary tract).

The urea cycle is the main pathway for detoxifing ammonia and it can be defective due to an inherited enzyme deficiency resulting in accumulated toxic metabolites. A common feature amoung urea cycle diseases is a defect in the elimination of ammonium by the liver, leading to hyperammonemia.[1,4,5] Hyperammonemia is a life-threatening condition which can affect patients at any age. Elevations of ammonia in plasma indicate its increased production and/or decreased detoxification. Ammonium toxicity can lead to neurological problems and brain damage. Better understanding of the pathophysiology of ammonium toxicity to the brain will allow the development of new strategies for neuroprotection.

About This Assay

Cayman's Urea Assay provides a convenient method for detecting urea in plasma, serum, and urine. In this assay, urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Ammonia reacts with the detector resulting in a fluorescent product. Fluorescence is analyzed with an excitation wavelength of 405-415 nm and an emission wavelength of 470-480 nm.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Potassium Phosphate Buffer (500 mM; pH 7.0) - (Item No. 700621)**

   The vial contains 5 ml of 500 mM potassium phosphate buffer (pH 7.0). Dilute the contents of the vial with 45 ml of HPLC-grade water. This final Buffer (50 mM potassium phosphate, pH 7.0) is used in the assay and for diluting reagents. When stored at 4°C, this diluted Buffer is stable for three months.

2. **Urease Assay Reagent - (Item No. 700622)**

   Each vial contains a lyophilized powder of urease. Reconstitute the contents of the vial with 1.2 ml of diluted Buffer. One vial of urease is enough enzyme to assay 60 wells. Reconstitute the additional vial if assaying the entire plate. The reconstituted enzyme is stable for five days at -20°C.

3. **Urea Standard - (Item No. 700623)**

   Each vial contains a lyophilized powder of urea. Reconstitute the contents of the vial with 1 ml of diluted Buffer to yield a 5 mM stock solution. It is ready to prepare the standard curve (see page 12). The reconstituted urea is stable for three days at -20°C.

4. **Urea Ammonia Detector - (Item No. 700624)**

   Each vial contains a lyophilized powder of ammonia detector. Reconstitute the contents of the vial with 600 µl of ethanol. One vial of detector is enough reagent to assay 60 wells. Reconstitute the additional vial if assaying the entire plate. The reconstituted reagent is stable for one day at -20°C.

5. **Ethanol Assay Reagent - (Item No. 700566)**

   The vial contains 2 ml of ethanol. It is ready to use in the assay.

Sample Preparation

NOTE: Blood/erythrocytes interfere with the assay and should not be used.

Plasma

Typically, normal human plasma has urea concentrations in the range of 2-7 mM.

1. Collect blood using an anticoagulant such as heparin or EDTA.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 25°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer.
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 1:10-1:20 with diluted Buffer before assaying.

Serum

Typically, normal human serum has urea concentrations in the range of 2-7 mM.

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 25°C. Pipette off the top yellow serum layer without disturbing the white buffy layer.
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 1:10-1:20 with diluted Buffer before assaying.

Urine

Typically, normal human urine has urea concentrations in the range of 50-300 mM.

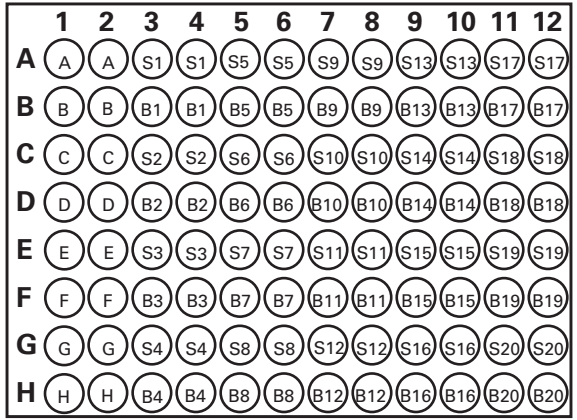
1. Collect urine in a clean container.
2. If not assaying the same day, freeze at -80°C. The urine sample will be stable for one month while stored at -80°C.
3. Dilute urine 1:200-1:400 with diluted Buffer before assaying.

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.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a urea standard curve in duplicate has to be assayed with the samples. We suggest that each sample be assayed at least in duplicate in the presence and absence of urease. A typical layout of standards, samples, and sample backgrounds to be measured in duplicate is given below in Figure 1. We suggest you record the contents of each well on the template sheet provided (see page 18).



A-H = Standards
S1-S20 = Sample Wells
B1-B20 = Sample Background Wells

Figure 1. 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 200 µl in all the wells.
- All reagents except urease 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 at least in duplicate (triplicate preferred).
- The assay is performed at room temperature.
- Monitor the fluorescence with an excitation wavelength of 405-415 nm and an emission wavelength of 470-480 nm.

Standard Preparation

Take eight clean glass test tubes or chemically resistant plastic and mark them A-H. Add the amount of reconstituted Urea (5 mM) and diluted Buffer to each tube as described in Table 1. The diluted Standards are stable for eight hours at 4°C.

Tube	Urea Standard (µl)	Buffer (µl)	Final Concentration (mM)
A	0	1,000	0
B	10	990	0.05
C	20	980	0.1
D	40	960	0.2
E	80	920	0.4
F	120	880	0.6
G	160	840	0.8
H	200	800	1.0

Table 1. Preparation of urea standards

Performing the Assay

1. **Standard Wells -** add 150 µl of diluted Buffer and 20 µl of standard (tubes A-H) per well in the designated wells on the plate (see **Sample plate format**, Figure 1, page 10).
2. **Sample Wells -** add 150 µl of diluted Buffer and 20 µl of sample to at least two wells.
3. **Sample Background Wells -** add 170 µl of diluted Buffer and 20 µl of sample to at least two wells.
4. Initiate the reactions by adding 20 µl of Urease to all standard and sample wells. DO NOT add to sample background wells.
5. Cover the plate with the plate cover and incubate the plate for 10 minutes at room temperature.
6. Remove the plate cover and add 10 µl of Ammonia Detector to every well being used including sample background wells.
7. Cover the plate with the plate cover and incubate the plate for 15 minutes at room temperature.
8. Remove the plate cover and read fluorescence using an excitation wavelength of 405-415 nm and an emission wavelength of 470-480 nm.

ANALYSIS

Calculations

1. Determine the average fluorescence of each standard, sample, and sample background.
2. Subtract the fluorescence value of the standard A from itself and all other standards. 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 urea from Table 1. See Figure 2, on page 15, for a typical standard curve.
4. Subtract the sample background fluorescence value from the sample value. This is the corrected sample fluorescence value (CSF).
5. Calculate the concentration of urea for each sample using the equation below.

$$\text{Urea (mM)} = \left[\frac{\text{CSF - (y-intercept)}}{\text{Slope}}\right] \times \text{Sample dilution}$$

NOTE: Urea values from urine samples can be standardized using Cayman's Creatinine Assay Kit (Item No. 500701).

Performance Characteristics

Precision:

When a series of 16 human plasma and urine mesurements were performed on the same day, the intra-assay coefficient of variation was 2.1 and 1.7%, respectively. When a series of 16 human plasma and urine mesurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 2.4 and 2.0%, respectively.

Sensitivity:

The limit of detection for the assay is 0.05 mM (±0.025 mM) urea.

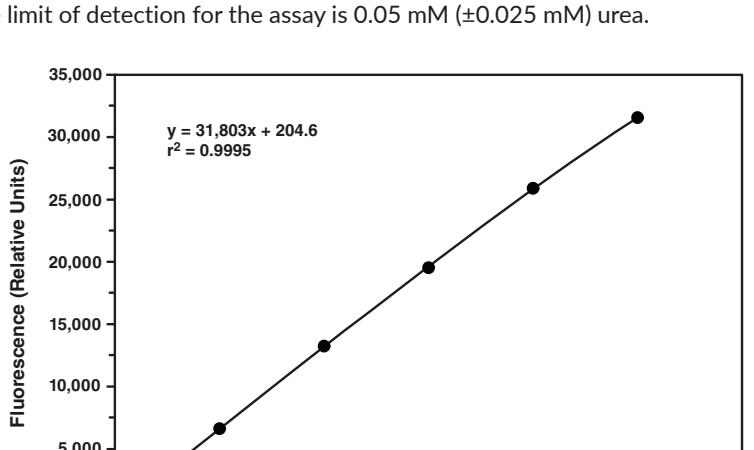


Figure 2. Urea Standard Curve

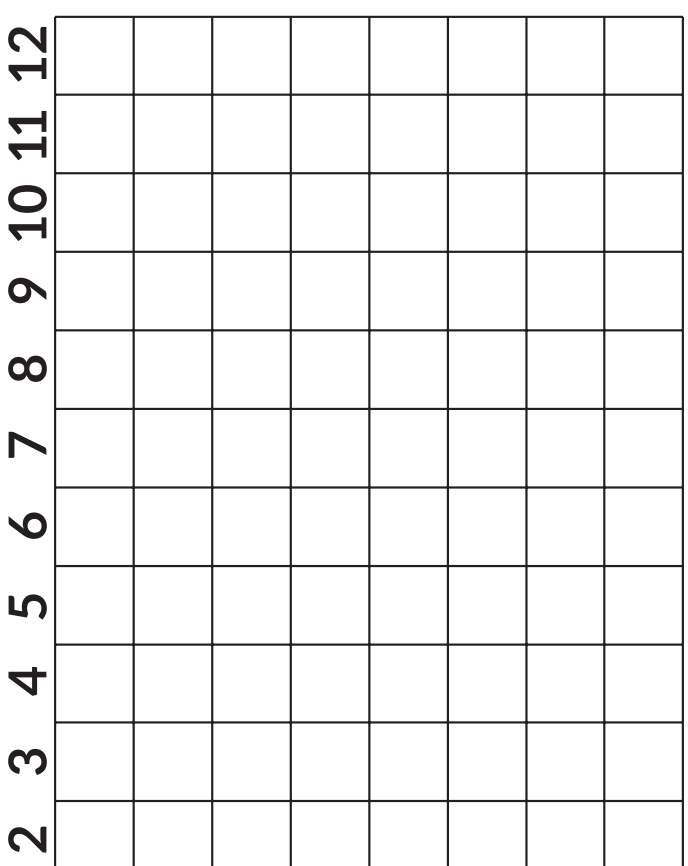
RESOURCES

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 detected above background in the sample wells	Sample was too dilute	Re-assay the sample using a lower dilution
The fluorometer exhibited 'MAX' values for the well(s)	The GAIN setting is too high	Reduce the GAIN and re-read
The fluorescence of the sample wells were higher than the last standard	Sample was too concentrated	Re-assay the sample using a higher dilution

References

1. Walker, V. Ammonia toxicity and its prevention in inherited defects of the urea cycle. *Diabetes, Obesity and Metabolism* **11**, 823-835 (2009).
2. Edwards, A. Modeling transport in the kidney: Investigating function and dysfunction. *Am. J. Physiol. Renal Physiol.* **298**, F475-F484 (2009).
3. Sands, J.M. and Layton, H.E. The physiology of urinary concentration: An update. *Semin. Nephrol.* **29(3)**, 178-195 (2009).
4. Braissant, O. Current concepts in the pathogenesis of urea cycle disorders. *Mol. Genet. Metab.* **100**, S3-S12 (2010).
5. Häberle, J. Clinical practice: The management of hyperammonemia. *Eur. J. Pediatr.* **170**, 21-34 (2011).



NOTES

Warranty and Limitation of Remedy

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