AAT Bioquest®

PRODUCT INFORMATION SHEET

Amplite™ Colorimetric Urea Quantitation Kit *Blue Color*

Catalog number: 10058
Unit size: 200 Tests

Component	Storage	Amount
Component A: Assay Enzyme Mix	Freeze (<-15 °C), Minimize light exposure	1 vial (lyophilized powder)
Component B: Assay Buffer I	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component C: Assay Buffer II	Freeze (<-15 °C), Minimize light exposure	1 bottle (10 mL)
Component D: Urea Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (1M, 100 µL)

OVERVIEW

Urea is the final degradation product of protein and amino acid metabolism in animals. It is produced in liver, secreted by kidney and excreted through urine. The determination of urea is very useful test in clinical laboratory to monitor health status. The Blood Urea Nitrogen (BUN) test is a measure of the amount of nitrogen in the blood in the form of urea and is primarily used, along with the creatinine test to evaluate kidney function, helping diagnose kidney diseases. Our Amplite™ Colorimetric Urea Assay Kit provides a simple and sensitive colorimetric method for the quantitation of urea concentration in biological samples such as serum, plasma and urine, etc. The assay is based on an enzyme-coupled reaction of urea in the assay buffer, and finally produces a blue colored product. The intensity of color produced is proportional to the concentration of urea in the sample, which can be measured colorimetrically at 660-670 nm. This Amplite™ Colorimetric Urea Assay Kit provides a simple assay to detect as little as 10 µM urea in a 150 µL assay volume. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

AT A GLANCE

Protocol summary

1. Prepare urea standards or test samples (50 µL)
2. Add urea working solution (50 µL)
3. Incubate at room temperature or 37°C for 30 - 60 min
4. Add Assay Buffer II
5. Read Absorbance at 665 nm

Important Thaw all the kit components to room temperature before starting the experiment.

KEY PARAMETERS

Instrument: Absorbance microplate reader
Absorbance: 665 nm
Recommended plate: Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Assay Enzyme Mix stock solution (100X):
Add 100 µL of ddH_2O into the vial of Assay Enzyme Mix (Component A) to make 100X Assay Enzyme Mix stock solution.

PREPARATION OF STANDARD SOLUTION

Urea standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10058

Add 1 µL of 1.0 M Urea Standard (Component D) to 999 µL of DPBS to generate 1.0 mM standard urea solution (US7). Take 1.0 mM urea standard solution to perform 1:3 serial dilutions to get remaining urea standard solutions (US6 - US1).

PREPARATION OF WORKING SOLUTION

Add 50 µL of reconstituted Assay Enzyme Mix stock solution (100X) into 5 mL Assay Buffer I (Component B) to make urea working solution.

Note The urea working solution should be used promptly and kept from light. The assay sensitivity will be decreased with longer storage time. Fresh urea working solution is recommended for the best result.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of urea standards and test samples in a clear bottom 96-well microplate. US= Urea Standards (US1 - US7, 1 to 1000 µM), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
US1	US1	...	...
US2	US2	...	...
US3	US3		
US4	US4		
US5	US5		
US6	US6		
US7	US7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
US1 - US7	50 µL	Serial Dilutions (1 to 1000 µM)
BL	50 µL	DPBS
TS	50 µL	test sample

1. Prepare urea standards (US), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of urea working solution to each well of urea standard, blank control, and test samples to make the total urea assay volume of 100 µL/well. For a 384-well plate, add 25 µL of urea working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction for 30 - 60 minutes at room temperature or 37°C, protected from light.

4. Add 50 µL of Assay Buffer II (Component C) to each well so that the total assay volume is 150 µL/well. For a 384-well plate, add 25 µL Assay Buffer II

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(Component C) to each well, for a total assay volume of 75 µL/well.

5. Incubate at room temperature for 10 - 15 minutes, and monitor the absorbance increase at 660 - 670 nm using an absorbance microplate reader.

   Note The color turns to yellow after Assay Buffer II (Component C) is added, and the wells with urea standard or samples will show blue-green color after incubation. The intensity of the color will reach the maximum in 15 - 30 minutes, and is proportional to the concentration of urea.

   Note The final color is stable for ~1 hour in room temperature and the color intensity will decrease with time.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Absorbance) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Urea samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

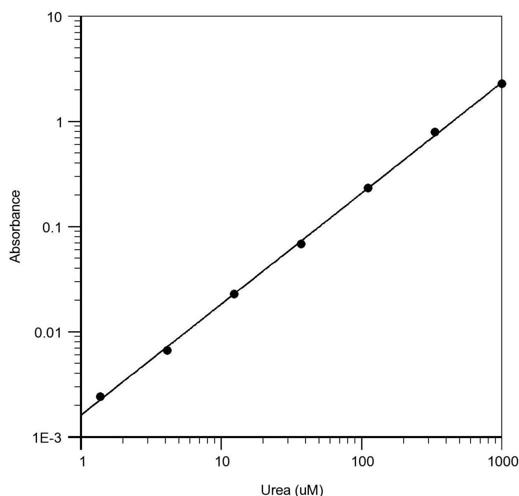

Figure 1. Urea dose response in a 96-well clear bottom plate using a SpectraMax microplate reader (Molecular Devices) measured with Amplite™ Colorimetric Urea Assay Kit.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

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