Amplite[™] Colorimetric Urea Quantitation Kit *Blue Color*

Ordering Information:	Storage Conditions:	Instrument Platform:
Product Number: 10058 (200 assays)	Keep in -20°C and avoid light	Absorbance microplate readers

Introduction

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 AmpliteTM 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 AmpliteTM Colorimetric Urea Assay Kit provides a simple assay to detect as little as 10 μ M urea in a 150 μ L 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.

Kit Key F	eatures
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Sensitive:	The kit detects as low as 10 µM urea in solution.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Assay Enzyme Mix	1 vial (lyophilized powder)
Component B: Assay Buffer I	1 bottle (10 mL)
Component C: Assay Buffer II	1 bottle (10 mL)
Component D: Urea Standard	1 vial (1M, 100 μL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare urea standards or test samples (50 μ L) \rightarrow Add Assay Reaction Mixture I (50 μ L) \rightarrow Incubate at room temperature or 37 °C for 30-60 min \rightarrow Add Assay Buffer II \rightarrow Read Absorbance at 665 nm

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

1. Prepare serial dilutions of urea (0 to 1mM) solutions:

- 1.1 Add 1µL of 1.0 M Urea Standard (Component D) to 999 µL DPBS to generate 1.0 mM standard urea solution.
- 1.2 Take 300 μL of 1.0 mM standard to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1, and 0 μM standard urea solutions.
- 1.3 Add urea standards and urea containing test samples into a 96-well clear bottom microplate as described in Tables 1 and 2.

 Table 1. Layout of urea standards and test samples in a clear bottom 96-well microplate:

BL	BL	TS	TS	 			
US 1	US 1			 			
US 2	US 2						
US 3	US 3						
US 4	US 4						
US 5	US 5						
US 6	US 6						
US 7	US 7						

Note: US= Urea Standards, BL=Blank Control, TS=Test Samples.

Table 2. Reagent composition for each well:

Urea Standard	Blank Control	Test Sample
Serial dilutions*: 50 µL	DPBS: 50 µL	50 μL

*Note: Add the serially diluted urea standards from 1 to 1000 µM into wells from US1 to US7 in duplicate.

2. Prepare Assay Reaction Mixture I:

- 2.1 Add 100 μ L of ddH₂O into the vial of Assay Enzyme Mix (Component A) to make 100X Assay Enzyme Mix solution.
- 2.2 Add 50 μL reconstituted Assay Enzyme Mix solution into 5 mL Assay Buffer I to make Assay Reaction Mixture I. Note 1: The Assay reaction mixture I should be used promptly and kept from light. The assay sensitivity will be decreased with longer storage time. The fresh Assay Enzyme Mixture I is recommended for the best result. Note 2: Any remaining 100X enzyme mix solution (from Step 2.1) needs to be aliquot and frozen at -20°C, avoid repeated freeze-thaw cycles.

3. Run urea assay:

- 3.1 Add 50 μL of Assay Reaction Mixture I (from Step 2.2) to each well of the urea standard, blank control, and test samples (see Step 1.3) so that the total assay volume is 100 μL/well. Note: For a 384-well plate, add 25 μL sample, 25 μL of assay reaction mixture I per well.
- 3.2 Incubate the reaction for 30-60 minutes at room temperature or 37°C, protected from light.
- 3.3 Add 50 μL of Assay Buffer II (Component C) to each well so that the total assay volume is 150 μL/well. *Note: For a 384-well plate, add 25 μL Assay Buffer II (Component C) to each well.*
- 3.4 Incubate at room temperature for 10-15 minutes, and monitor the absorbance increase at 660-670 nm using an absorbance microplate reader.

Note 1: 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 15-30minutes, and is proportional to the concentration of urea.

Note 2: The final color is stable for ~1 hour in room temperature and the color intensity will decrease with time.

Data Analysis

The absorbance in blank wells (with DPBS only) is used as a control, and is subtracted from the values for those wells with urea reactions. The typical data are shown in Figure 1 (urea standard curve). *Note: The absorbance background is subtracted from the absorbance intensity value of the wells for each data point.*

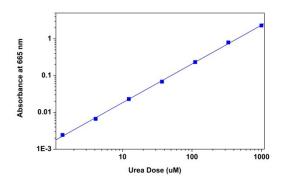


Figure 1. Urea dose response in a 96-well clear bottom plate using a Spectrum Max microplate reader (Molecular Devices) measured with AmpliteTM Colorimetric Urea Assay Kit. As low as 10 μ M urea can be detected (n=3) in 15 minutes incubation after Assay Buffer II is added.

References

- 1. Gibb, Bruce C. (2009). "Teetering towards chaos and complexity". *Nature Chemistry* (Nature Publishing Group) 1: 17–18.
- 2. Marsh, K. L., G. K. Sims, and R. L. Mulvaney. 2005. Availability of urea to autotrophic ammonia-oxidizing bacteria as related to the fate of 14C- and 15N-labeled urea added to soil. Biol. Fert. Soil. 42:137-145.
- 3. Baumgartner, M., M. Flöck, P. Winter, W. Lu, and W. Baumgartner. 2005. Evaluation of flow injection analysis for determination of urea in sheep's and cow's milk. Acta Veterinaria Hungarica. 50 (3): 263-271.
- 4. Greenan, N. S., R.L. Mulvaney, and G.K. Sims. 1995. A microscale method for colorimetric determination of urea in soil extracts. Communications in Soil Science and Plant Analysis. 26:2519-2529.
- 5. Godfrey, Peter; Brown, Ronald and Hunter, Andrew (1997). "The shape of urea". *Journal of Molecular Structure* 413-414: 405–414.

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