

Amplite™ Colorimetric Urea Quantitation Kit

Blue Color

Ordering Information:

Product Number: 10058 (200 assays)

Storage Conditions:

Keep in -20°C and avoid light

Instrument Platform:

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 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.

Kit Key Features

<i>Sensitive:</i>	The kit detects as low as 10 µM urea in solution.
<i>Continuous:</i>	Easily adapted to automation with no separation required.
<i>Convenient:</i>	Formulated to have minimal hands-on time.
<i>Non-Radioactive:</i>	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 µL) → Add Assay Reaction Mixture I (50 µL) → Incubate at room temperature or 37 °C for 30-60 min → Add Assay Buffer II → Read Absorbance at 665 nm

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

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.

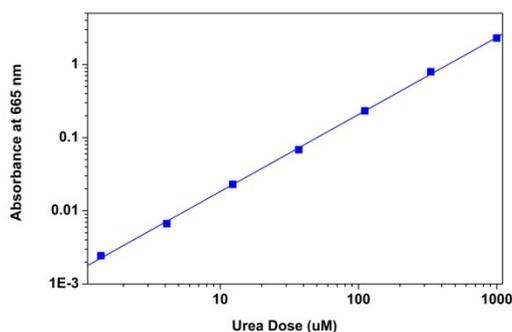


Figure 1. Urea dose response in a 96-well clear bottom plate using a Spectrum Max microplate reader (Molecular Devices) measured with Amplite™ Colorimetric Urea Assay Kit. As low as 10 µM urea can be detected (n=3) in 15 minutes incubation after Assay Buffer II is added.

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.

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.