Urea Assay Kit II (Colorimetric)

LS-K296-100 (100 Tests) • **See Storage Conditions Below**

Introduction

UREA, the major end product of protein catabolism in animals, is primarily produced in the liver and secreted by the kidneys. It is the primary vehicle for removal of toxic ammonia from the body. Urea determination is very useful for medical clinicians to assess kidney function of patients. In general, increased urea levels are associated with nephritis, renal ischemia, urinary tract obstruction, and certain extrarenal diseases (e.g. congestive heart failure, liver diseases, and diabetes). Decreased levels often indicate acute hepatic insufficiency, but may also result from over vigorous parenteral fluid therapy. This colorimetric urea (BUN) assay is based on urease catalyzed conversion of urea to ammonium and carbon dioxide. The pH of the reaction medium is monitored by a chromogen and the intensity of the reaction product at 557 nm is directly proportional to the urea concentration in the sample.

Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 1 mg/mL (0.17 mM) to 100 mg/mL (17 mM) urea in 96-well plate assay.
- Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 5 minutes. Room temperature assay. No 37°C heater is needed.
- High-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

Applications

- Urea in biological samples (e.g. plasma, serum, urine) and food/beverage samples (e.g. milk).

Components

Component	K296-100
	100 Tests
Reagent	10 mL
Urease	120 µL
Standard (200 mg/dL)	1 mL

Materials Not Supplied

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and plate reader.

Storage

The kit is shipped at room temperature. Store Reagent and Standard at 4°C upon receiving. Urease can be stored from -20°C to 4°C. For long-term storage, keep standard at -20°C. Shelf life: 6 months after receipt.

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Assay Procedure

Sample Preparation

Serum and Plasma can be assayed directly after centrifuging to remove any particulates (n=1).

Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 g. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant should then be diluted 5 -fold in distilled water (n=6.8).

Urine: Dilute 50-fold in distilled water (n=50). Urine samples do not require an internal standard.

Reagent Preparation

Vortex reagent or warm in a bath if there are any particulates. Equilibrate Reagent to room temperature. Briefly centrifuge other tubes before use.

Procedure

1. Samples. Samples require an internal standard and need three separate reactions: 1) sample plus standard, 2) sample alone and 3) sample blank. For the sample plus standard well, add 5 µL 200 mg/dL urea and 20 µL sample. For the sample and sample blank wells, add 5 µL dH_2O and 20 µL sample.
2. Urea Detection. Prepare enough working reagent (WR) for all samples plus standards and samples alone. For each reaction combine the following: 85 µL Reagent and 1 µL Urease.

   Add 80 µL WR to each sample plus standard and sample alone well.

   Add 80 µL Reagent (No Urease) to each sample blank well. Tap plate to mix briefly and thoroughly. Incubate 5 minutes at room temperature.
3. Read OD_{557nm} (550-565 nm).

Calculations

The sample urea concentration is computed as follows:

$$[\text{Urea}] = \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times \frac{[\text{Standard}]}{4} \times n \quad (\text{mg/dL})$$

$$= \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times 50 \times n \quad (\text{mg/dL})$$

where R_{SAMPLE}, R_{BLANK} and $R_{STANDARD}$ are OD readings of the Sample, Sample Blank, and the Sample plus Standard respectively. *n* is the sample dilution factor. The volume of the internal standard is 4× lower than the sample volume; thus, the internal standard concentration should be divided by 4.

Note: If the calculated urea concentration is greater than 50 mg/dL urea, dilute sample in distilled water and repeat the assay. Multiply the results by the dilution factor *n*.

Conversions: BUN (mg/dL) = [Urea] / 2.14. 1 mg/dL urea equals 167 µM, 0.001% or 10 ppm.

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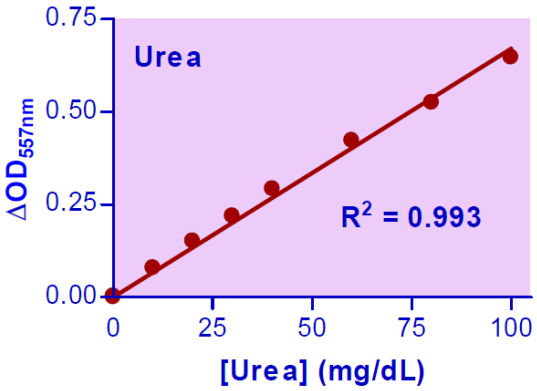
Sample Data



Standard Curve in 96-well plate assay in water.

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