Urea Assay Kit

Catalog Number KA7499

100 assays

Version: 01

Intended for research use only

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Introduction

Background

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.

Principle of the Assay

Urea Assay Kit 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.

✓ Applications

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

✓ Key Features

- Fast and sensitive. Linear detection range (20 µL sample): 1 mg/dL (0.17 mM) to 100 mg/dL (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 on HTS liquid handling systems for processing thousands of samples per day.

General Information

Materials Supplied

List of component

Component	Amount
Reagent	10 mL
Standard: (200 mg/dL)	1 mL
Urease	120 µL

Storage Instruction

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.

Materials Required but Not Supplied

- ✓ Pipetting devices
- ✓ Accessories (e.g. Multi-channel pipettor)
- ✓ Clear flat-bottom 96-well plates (e.g. Corning Costar)
- ✓ Centrifuge tubes
- ✓ Plate reader

Precautions for Use

- ✓ Reagents are for research use only.
- ✓ Normal precautions for laboratory reagents should be exercised while using the reagents.

Assay Protocol

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.

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. For information on how to run the assay with urine samples.

Assay Procedure

✓ Reaction Preparation:

1. Samples. Samples require an internal standard and need three separate reactions:
   - sample plus standard,
   - sample alone and
   - 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).

Data Analysis

Calculation of Results

The sample urea concentration is computed as follows:

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

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

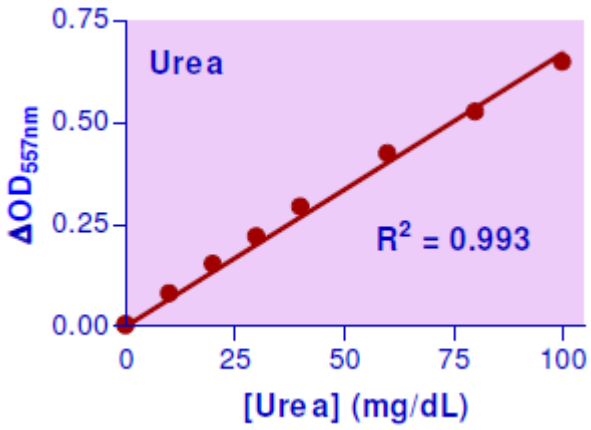
where R_{SAMPLE}, R_{BLANK} and $R_{STANDARD}$ are OD readings of the Sample, Sample Blank, and the Sample plus Standard respectively. n is the sample dilution factor. The volume of the internal standard is 4× lower than the sample volume; thus, the internal standard concentration should be divided by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

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

✓ Standard Curve in 96-well plate assay in water.



Resources

References

1. Kumar, H., et al (2000). A test strip for the estimation of urea in serum. Inidian J Clin Biochem. 15(2): 124-7.
2. Orsonneau, JL, et al (1992). Simply and sensitive determination of urea in serum and urine. Clin Chem. 38(5): 619-23.
3. Krysteva, M, et al (2003). Optical enzyme sensor for urea determination via immobilized pH indicator and urease onto transparent membranes. ScientificWorldJournal. 3: 585-92.

Plate Layout

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