Technical Bulletin

Urea Assay Kit III

Catalogue number MAK471

Product Description

Urea is the major end product of protein catabolism in animals. Urea 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 useful in assessing kidney function. In general, increased urea levels are associated with nephritis, renal ischemia, urinary tract obstruction, and certain extrarenal diseases (for example, congestive heart failure, liver diseases, and diabetes). Decreased levels often indicate acute hepatic insufficiency but may also result from over vigorous parenteral fluid therapy.

Simple, direct, and automation-ready procedures for measuring urea or Blood Urea Nitrogen (BUN) are popular in research and drug discovery. The Urea Assay Kit III is designed to directly measure urea in biological samples. In this assay, urease converts urea to ammonia and carbon dioxide. NADH is then converted to NAD+ in the presence of ammonia, α-ketoglutarate, and glutamate dehydrogenase. The decrease in optical density at 340 nm is directly proportional to the urea concentration in the sample.

The linear detection range of the kit is 50 to 1000 µM urea in the 96-well plate assay. The kit is suitable for detection of urea in biological samples such as plasma, serum, urine, bronchoalveolar lavage (BAL), and food/beverage samples such as milk.

Components

The kit is sufficient for 100 spectrophotometric assays in 96-well plates.

- Assay Buffer — 20 mL
  Catalogue Number MAK471A
- Enzyme — 120 µL
  Catalogue Number MAK471B
- Ketoglutarate — 120 µL
  Catalogue Number MAK471C
- Urease — 120 µL
  Catalogue Number MAK471D
- NADH Reagent — 1 Vial
  Catalogue Number MAK471E
- Standard (40 mM) — 400 µL
  Catalogue Number MAK471F

Equipment Required but Not Provided

- Pipetting devices and accessories (such as, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- Microcentrifuge capable of RC ≥14,000×g
- 1.5 mL microcentrifuge tubes

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Equilibrate all components to room temperature prior to use.

NADH Reagent: Reconstitute vial with 1 mL of purified water (final concentration is 10 mM). Unused reconstituted NADH reagent is stable for three weeks when stored frozen at -20 °C.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

1. Serum and plasma samples should be centrifuged to remove any particulates and then diluted 10-fold in purified water (DF = 10).
2. Urine should be diluted 500-fold in purified water (DF = 500).
3. Milk samples should be cleared by mixing 600 µL of milk with 100 µL of 6 N HCl. Centrifuge for 5 minutes at 14,000 × g at room temperature. Transfer 300 µL of supernatant into a clean tube and neutralize with 50 µL of 6 N NaOH. The neutralized supernatant should then be diluted 20 fold in purified water (DF = 27.2).
4. Avoid use of cell culture media containing phenol red. Other media can be assayed directly.
5. Samples should be clear and colorless with pH adjusted to 7 – 8.
6. Transfer 20 µL of each Sample into duplicate separate wells of a clear 96 well plate. One well will be used as the reaction Sample and the duplicate well the Sample Blank.

Standard Curve Preparation

1. Prepare a 1000 µM Urea Standard by mixing 15 µL of the 40 mM Urea Standard with 585 µL of purified water. Prepare Urea Standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of Urea Standard

Standard	1000 µM Urea Standard	Purified Water	Urea (µM)
1	100 µL	-	1000
2	60 µL	40 µL	600
3	30 µL	70 µL	300
4	-	100 µL	0

2. Mix well and transfer 20 µL of each Standard into separate wells of the plate.

Working Reagents

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 2.
   a. For each Urea Standard and Sample well, prepare 191 µL of Working Reagent.
   b. For each Sample Blank well, prepare 190 µL of Blank Working Reagent.

Table 2.
Preparation of Working Reagents

Reagent	Working Reagent	Blank Working Reagent
Assay Buffer	180 µL	180 µL
Enzyme	1 µL	1 µL
NADH Reagent	8 µL	8 µL
Ketoglutarate	1 µL	1 µL
Urease	1 µL	-

2. Add 180 µL of Working Reagent to each Standard and Sample well.
3. Add 180 µL of Blank Working Reagent to each Sample Blank well.
4. Tap the plate to mix.

Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Read optical density (OD) at 340 nm.

Results

1. Calculate ΔOD by subtracting the blank OD reading of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔOD against standard concentrations.
3. Determine the slope of the standard curve and calculate the urea concentration of the sample.

$$\text{Urea } (\mu M) = \frac{OD_{Blank} - OD_{Sample}}{\text{Slope } (\mu M^{-1})} \times DF$$

where:

OD_{Sample} = OD reading of Sample

OD_{Blank} = OD reading of Sample Blank

DF = Sample dilution factor (DF = 1 for undiluted samples)

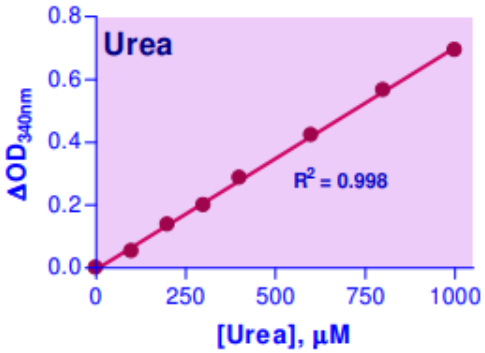
Conversions: 1000 µM urea equals 6 mg/dL or 60 ppm.

Urea BUN (mg/dL) = [Urea] (mg/dL) / 2.14

If the calculated urea concentration is higher than 1000 µM, dilute the sample in purified water and repeat the assay. Multiply result by the dilution factor (DF).

Figure 1.

Typical Urea Standard Curve



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