EnzyChrom™ Urea Assay Kit III (EUR3-100)

Quantitative Colorimetric Determination of Urea at 340 nm

DESCRIPTION

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.

Simple, direct and automation-ready procedures for measuring urea or blood urea nitrogen (BUN) are popular in research and drug discovery. BioAssay Systems' urea assay 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.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 50 to 1000 µM urea in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent and reading the absorbance after 30 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.

APPLICATIONS

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

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	20 mL	**Enzyme**:	120 µL
Ketoglutarate:	120 µL	**Urease:**	120 µL
NADH Reagent:	Dried	**Standard**:	400 µL

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life: 6 months after receipt, 3 weeks after reconstitution of dried NADH Reagent.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample preparation. *Serum and plasma* samples should be centrifuged to remove any particulates and then diluted 10-fold in dH_2O (n=10).

Urine should be diluted 500-fold in dH_2O (n=500).

Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 x g. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant should then be diluted 20-fold in dH_2O (n = 27.2).

Cell culture media containing phenol red should be avoided. Other media can be assayed directly (n=1).

Samples should be clear and colorless with pH adjusted to 7 - 8.

Reagent Preparation. Equilibrate all components to room temperature. Briefly centrifuge all tubes before opening. Reconstitute the NADH Reagent tube with 1 mL dH_2O (final 10 mM). Unused reconstituted NADH reagent is stable for three weeks when stored frozen at -20°C.

Colorimetric Procedure

1. *Standards and Samples*. Prepare a 1000 µM Urea Standard Premix by mixing 15 µL of the 40 mM Standard and 585 µL dH_2O. Dilute Standard as follows.

No	Premix + dH_2O	Vol (µL)	Urea (µM)
1	100 µL + 0 µL	100	1000
2	60 µL + 40 µL	100	600
3	30 µL + 70 µL	100	300
4	0 µL + 100 µL	100	0

Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate.

Transfer 20 µL of each sample into two separate wells, one serving as a sample blank well (R_{BLANK}) and one as a sample well (R_{SAMPLE}).

2. *Enzyme Reaction*. For each standard and sample well, prepare Working Reagent by mixing 180 µL Assay Buffer, 1 µL Enzyme, 8 µL reconstituted NADH Reagent, 1 µL Urease, and 1 µL Ketoglutarate. Prepare blank control reagent by mixing 180 µL Assay Buffer, 8 µL reconstituted NADH Reagent, 1 µL Enzyme, and 1 µL Ketoglutarate (***No Urease***).

   Add 180 µL Working Reagent to the *four Standards* and the *Sample* Wells. Add 180 µL Blank Control Reagent only to the *Sample Blank* Wells.

   Tap plate to mix. Incubate 30 min at room temperature.

3. Read OD_{340nm}.

CALCULATION

Subtract the standard values from the blank value (#4) and plot the ΔOD against standard concentrations. Determine the slope and calculate the Urea concentration of Sample,

$$[\text{Urea}] = \frac{OD_{BLANK} - OD_{SAMPLE}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Sample Blank, respectively. *n* is the sample dilution factor.

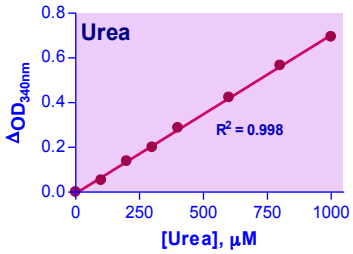
Note: if the calculated urea concentration is higher than 1000 µM, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor *n*.

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

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

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, and clear flat-bottom 96-well plates, and optical density plate reader for colorimetric assays.



LITERATURE

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