QuantiChrom™ Urea Assay Kit (DIUR-500)

Quantitative Colorimetric Urea Determination at 520nm

DESCRIPTION

Urea is primarily produced in the liver and secreted by the kidneys. Urea is the major end product of protein catabolism in animals. It is the primary vehicle for removal of toxic ammonia from the body. Urea determination is very useful for the medical clinician 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 indicate acute hepatic insufficiency or may result from over-vigorous parenteral fluid therapy.

Simple, direct and automation-ready procedures for measuring urea concentration in biological samples are becoming popular in Research and Drug Discovery. BioAssay Systems' urea assay kit is designed to measure urea directly in biological samples without any pretreatment. The improved Jung method utilizes a chromogenic reagent that forms a colored complex specifically with urea. The intensity of the color, measured at 520nm, is directly proportional to the urea concentration in the sample. The optimized formulation substantially reduces interference by substances in the raw samples.

KEY FEATURES

Sensitive and accurate. Use 5 µL samples. Linear detection range 0.006 mg/dL (1µM) to 100 mg/dL (17mM) urea in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of a single working reagent and incubation for 5 min. Can be readily automated as a high-throughput assay for thousands of samples per day.
Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.
Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on raw biological samples i.e., in the presence of lipid and protein.

APPLICATIONS:

Direct Assays: urea in serum, plasma, urine, milk etc.
Drug Discovery/Pharmacology: effects of drugs on urea metabolism.
Environment: urea determination in waste water, soil etc.

KIT CONTENTS (500 tests in 96-well plates)

Reagent A: 50 mL Reagent B: 50 mL
Urea standard: 1 mL 50 mg/dL

Storage conditions. All components are stable at 4°C for 12 months.

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

Reagent Preparation:

Equilibrate reagents to room temperature. Prepare enough working reagent by combining equal volumes of Reagent A and Reagent B, shortly prior to assay. Use working reagent within 20 min after mixing.

Procedure using 96-well plate:

1. Samples. Serum and plasma samples can be assayed directly (n = 1). Urine samples should be diluted 5-fold in distilled water prior to assay (n = 5).

   Transfer 5 µL water (blank), standard (50mg/dL) and samples in duplicate into wells of a clear bottom 96-well plate.

2. Add 200 µL working reagent and tap lightly to mix.

3. Incubate 10 min to 20 min at room temperature and read optical density at 470 - 550 nm (peak absorbance at 520nm).

Procedure using cuvette:

Prepare diluted samples as described for 96-well plate assay. Transfer 20 µL water, standard and diluted samples to appropriately labeled tubes. Add 1000 µL working reagent and tap lightly to mix. Incubate 10 min to 20 min and read optical density at 520nm.

CALCULATION

urea concentration of the sample is calculated as

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

OD_{SAMPLE}, OD_{BLANK} and $OD_{STANDARD}$ are OD_{520nm} values of sample, standard and water, respectively. n is the dilution factor.
Conversions: **Urea** (mg/dL) = **BUN** (mg/dL) x 2.14.
1 mg/dL urea equals 167 µM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. 5 µL).

Procedure using 96-well plate:

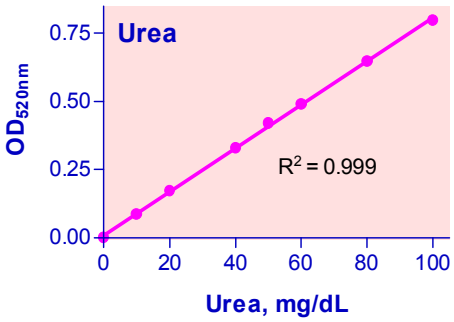
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD 520nm.

EXAMPLES

Biological samples were assayed in duplicate using the 96-well protocol. The urea concentration (mg/dL) was 12.5 ± 0.9 for Commercial 2% reduced fat milk (Kirkland), 35.7 ± 0.1 for Invitrogen fetal bovine serum, 22.1 ± 0.9 for human serum, 22.3 ± 0.2 for human plasma, 31.8 ± 1.1 for rat serum, 42.6 ± 0.1 for rat plasma and 103 ± 1 for a fresh human urine sample.



Calibration curve in 96-well plate

LITERATURE

1. Jung D et al (1975). New Colorimetric reaction for end-point, continuous-flow, and kinetic measurement of urea. Clin Chem. 21(8):1136-1140.

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3. Levinson SS (1978). Kinetic centrifugal analyzer and manual determination of serum urea nitrogen, with use of *o*-phthaldialdehyde reagent. Clin Chem. 24(12):2199-2202.