

User's Manual and Instructions

Urea Assay Kit (Z5030016)

Quantitative Colorimetric Urea Determination

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 or blood urea nitrogen BUN in biological samples are becoming popular in Research and Drug Discovery. Biochain's 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 μ L samples. Linear detection range 0.08 mg/dL (13 μ M) 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 20 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, cell/tissue culture, bronchoalveolar lavage (BAL) etc.

Drug Discovery/Pharmacology: effects of drugs on urea metabolism.

Environment: urea determination in wastewater, 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. The kit is shipped at room temperature. Store all components at 2-8°C. For long-term storage, keep standard at -20°C. Shelf life: at least 6 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. Serum and plasma samples can be assayed directly ($n = 1$). Urine samples should be diluted 50-fold in distilled water prior to assay ($n = 50$). Transfer 5 μ L water (blank), 5 μ L standard (50mg/dL) and 5 μ L samples in duplicate into wells of a clear bottom 96-well plate.

For low urea samples (< 5 mg/dL), e.g. issue/cell extract, culture medium, BAL etc, transfer 50 μ L water (blank), 50 μ L 5 mg urea/dL (the 50 mg/dL standard diluted in water) and 50 μ L samples in duplicate into separate wells.

2. Add 200 μ L working reagent and tap lightly to mix.

3. Incubate 20 min (50 min for low urea samples) at room temperature.

Read optical density at 520nm. For low urea samples, read OD at 430nm.

Procedure using cuvette:

Prepare samples as described for 96-well plate assay. Transfer 20 μ L water, standard (50 mg/dL) and samples to appropriately labeled tubes. For low urea samples, use 5 mg/dL standard and 200 μ L instead of 20 μ L. Add 1000 μ L working reagent and tap lightly to mix. Incubate 20 min (50min) and read OD_{520nm} (OD_{430nm}).

CALCULATION

Urea concentration (mg/dL) of the sample is calculated as

$$[\text{Urea}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{BLANK}}} \times n \times [\text{STD}] \text{ (mg/dL)}$$

OD_{SAMPLE}, OD_{BLANK} and OD_{STANDARD} are OD values of sample, standard and water, respectively. n is the dilution factor. [STD] = 50 (or 5 for low urea samples), urea standard concentration (mg/dL).

Conversions: BUN (mg/dL) = [Urea] / 2.14.

1 mg/dL urea equals 167 μ M, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. 5 μ L).

Procedure using 96-well plate:

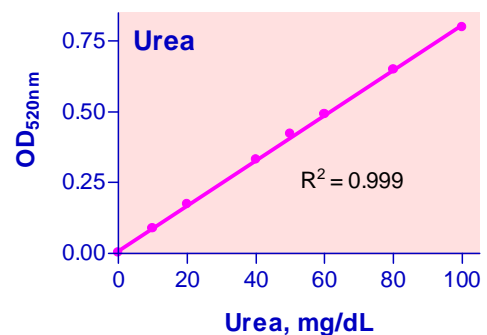
Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

Spectrophotometer and cuvetts for measuring OD_{520nm} and OD_{430nm}.

EXAMPLES

Biological samples were assayed in duplicate using the 96-well protocol. The urea concentration (mg/dL) was 12.5 \pm 0.9 for Commercial 2% reduced fat milk (Kirkland), 35.7 \pm 0.1 for Invitrogen fetal bovine serum, 22.1 \pm 0.9 for human serum, 22.3 \pm 0.2 for human plasma, 31.8 \pm 1.1 for rat serum, 42.6 \pm 0.1 for rat plasma and 1501 \pm 52 for a fresh human urine sample, 0.21 \pm 0.03 in a human BAL sample, 0.15 to 2.7 mg/dL in cell culture.



Standard Curve in 96-well plate assay

LITERATURE

- Ji, H., Bachmanov, A.A. (2007). Differences in postingestive metabolism of glutamate and glycine between C57BL/6ByJ and 129P3/J mice. *Physiol Genomics* 31(3):475-82.
- Snykers, S. et al (2007) Chromatin remodeling agent trichostatin A: a key-factor in the hepatic differentiation of human mesenchymal stem cells derived of adult bone marrow. *BMC Dev Biol.* 7:24.
- Zeng, L. et al (2006). Multipotent adult progenitor cells from swine bone marrow. *Stem Cells* 24:2355-2366.