Urea Assay Kit

Urea Assay Kit is a detection kit for the quantification of Urea in serum, plasma, urine, milk, cell culture supernatant, cell/tissue lysate and bronchoalveolar lavage.

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Package: 100 tests

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INTRODUCTION

Urea, also known as carbamide, is an organic compound with chemical formula CO(NH2)2. This amide has two –NH2 groups joined by a carbonyl (C=O) functional group.

Urea serves an important role in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. It is a colorless, odorless solid, highly soluble in water, and practically non-toxic (LD50 is 15 g/kg for rats). Dissolved in water, it is neither acidic nor alkaline. The body uses it in many processes, most notably nitrogen excretion. The liver forms it by combining two ammonia molecules (NH3) with a carbon dioxide (CO2) molecule in the urea cycle. Urea is widely used in fertilizers as a source of nitrogen (N) and is an important raw material for the chemical industry.

Friedrich Wöhler's discovery, in 1828, that urea can be produced from inorganic starting materials, was an important conceptual milestone in chemistry. It showed, for the first time, that a substance, previously known only as a byproduct of life, could be synthesized in the laboratory, without biological starting materials, thereby contradicting the widely held doctrine vitalism, which stated that only living things could produce the chemicals of life. [Wikipedia: Urea]

PRINCIPLE OF THE ASSAY

This Urea Activity Assay Kit provides a Simple, direct and automation-ready procedures for measuring Urea concentrations in s serum, plasma, urine, milk, cell culture supernatant, cell/tissue lysate and bronchoalveolar lavage. This Urea Activity Assay Kit is designed to use an improved Jung method measure Urea directly in samples without any pretreatment. It utilizes a specific color reaction with Urea. The absorbance at 520 nm is directly proportional to Urea concentration in the sample. The concentration of Urea in the sample is then determined by comparing the signals of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Urea Reagent A	12 ml (Ready to use)	4°C
Urea Reagent B	12 ml (Ready to use)	4°C
Standard (50 mg/dL)	0.5 ml	-20°C

The kit is shipped on ice. Store Urea Reagent at 4°C and store the standard at -20°C upon receipt. Shelf life of 12 months after receipt.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 520 nm.
- Flat bottomed 96-well microplate
- Pipettes and pipette tips
- Deionized or distilled water
- Boiling water bath or heat block.
- 1.5-mL centrifuge tubes
- Tube holder

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Briefly spin down the reagents before use.
- It is recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- All reagents should be warmed to room temperature before use.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 4°C. Collect serum and assay immediately or aliquot & store samples at -80°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 4°C within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -80°C. Avoid repeated freeze-thaw cycles.

Tissue lysate. Weight 20-100 mg of tissue and add 200-1000 µl of ice-cold PBS. Lysis samples by homogenization (10-20 passes in a Dounce homogenizer) or by sonication on ice. The degree of tissue lysis can be checked under a microscope. Then centrifuge at 14,000 rpm for 10 min at 4°C. Use clear supernatant for assay. Collect Samples and assay immediately or aliquot & store samples at -80°C. Avoid repeated freeze-thaw cycles.

Cell/tissue lysate – Add 400 µl of ice-cold PBS in 2 × 10^6 cells. Lysis samples by homogenization or by sonication on ice. The degree of cell lysis can be checked under a microscope. Then centrifuge at 14,000 rpm for 10 min at 4°C. Use clear supernatant for assay. Collect Samples and assay immediately or aliquot & store samples at -80°C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants or other liquid samples - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at -80°C. Avoid repeated freeze-thaw cycles.

Note:

1. Samples should be clear and free of particles or precipitates.
2. Avoid using haemolytic, icteric or lipaemic samples.
3. It is recommended to do pre-test to determine the suitable dilution factor. Choose a dilution with the readings in the linear range of the standard curve for further assays.

REAGENT PREPARATION

- Standard:

  - For *serum, plasma and urine* samples, add **5 µl** of the standard stock (50 mg/dL) in the appropriate wells directly.

  - To determine low Urea concentrations (<5 mg/dL, e.g. tissue/cell extract, BAL etc.,), dilute the standard at 10X dilution with distilled water to 5 mg/dL. Add **50 µl** of diluted standard (5 mg/dL) in the appropriate wells.

  - For culture medium samples, since culture medium containing phenol red, dilute the standard at 10X dilution with culture medium to 5 mg/dL. Add **50 µl** of diluted standard (5 mg/dL) in the appropriate wells.

- Samples:

  - *Serum and plasma samples* can be assayed directly (dilution factor n = 1).

  - *Urine* samples should be diluted 50-fold in distilled water prior to assay (dilution factor n = 50).

  - To determine *samples with low Urea concentrations (< 5 mg/dL, e.g. tissue/cell extract, BAL etc)*, add **50 µL** of sample a in the appropriate wells.

  - It is recommended to do pre-test to determine the suitable dilution factor. Choose a dilution with the readings in the linear range of the standard curve for further assays.

- **Working reagent:** Mix equal volumes of Urea Reagent A and Reagent B 20 minutes before use, mix well. Mix only needed volume for once. It is suggested using the working reagent mixture within 20 min after mixing.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use, each vial should be mixed thoroughly without foaming and briefly centrifuge tubes prior to use.

1. Add samples:

   1.1 Add **5 µL** of Serum and plasma samples or 50X diluted Urine sample in the appropriate wells. Add **5 µL** of distilled water in blank wells.

   1.2 *For low Urea concentrations samples (< 5 mg/dL e.g. tissue/cell extract, BAL etc)*, add **50 µL** of samples in the appropriate wells. Add **50 µL** of distilled water in blank wells.

   Note: For culture medium containing phenol red, add 50 µL of medium in blank well.

2. Add standard:

   2.1 For serum, plasma and urine samples, add **5 µL** of **standard stock (50 mg/dL)** in standard wells.

   2.2 For *low Urea concentrations samples (< 5 mg/dL e.g. tissue/cell extract, BAL etc)*, add **50 µL** of **10X diluted standard (5mg/dL)** in standard wells.

3. Add **200 µL** of **Working Reagent** in each well.
4. Gently tap the plate to ensure **thorough mixing**.
5. Incubate the plate at **RT for 20 min**. (For low Urea concentrations samples Incubate the plate at **RT for 50 min)**
6. Read the OD with a microplate reader at **520 nm** (For low Urea concentrations samples read the OD with a microplate reader at **430 nm)** immediately.

Summary:

A. For serum, plasma and urine

	Sample	Standard	Blank
Sample	5 µl	-	-
Distilled water	-	-	5 µl
Standard stock (50 mg/dL)	-	5 µl	-
Working Reagent	200 µl	200 µl	200 µl
A Gently tap the plate to ensure thorough mixing.			
Incubate the plate at RT for 20 min.			
Read the OD at 520 nm immediately.			

B. For low Urea concentrations samples (< 5 mg/dL)

	Sample	Standard	Blank
Sample	50 µl	-	-
Distilled water (medium)	-	-	50 µl
Diluted Standard (5 mg/dL)	-	50 µl	-
Working Reagent	200 µl	200 µl	200 µl
A Gently tap the plate to ensure thorough mixing.			
Incubate the plate at RT for 50 min.			
Read the OD at 430 nm immediately.			

Note: Some plasma samples will form precipitates after the addition of the working reagent. If this is the case, we recommend to perform the reaction in a microtube centrifuge at maximum speed for five minutes and use the clear supernatant for the measurement.

CALCULATION OF RESULTS

1. Urea concentration of the Samples are calculated as follows:

   [Urea] (mg/dL)=

   [(OD $_{SAMPLE}$ –OD $_{BLANK}$) / (OD $_{STANDARD}$ –OD $_{BLANK}$)] x n x (Standard Conc.)

   Note:

   a. OD $_{SAMPLE}$, OD $_{BLANK}$, OD $_{STANDARD}$ are OD values of sample, blank and standard.

   b. n is the dilution factor.

   c. Standard Conc. = 50 mg/dL (or 5 mg/dL).

2. Conversions: BUN (mg/dL) = [Urea] / 2.14.
3. 1 mg/dL urea equals 167 µM, 0.001% or 10 ppm.
4. Biological samples were assayed and the urea concentrations were as below (mg/dL):

   a. Commercial 2% reduced fat milk: 12.5 ± 0.9

   b. Invitrogen fetal bovine serum: 35.7 ± 0.1

   c. Human serum: 22.1 ± 0.9

   d. Human plasma: 22.3 ± 0.2

   e. Rat serum: 31.8 ± 1.1

   f. Rat plasma: 42.6 ± 0.1

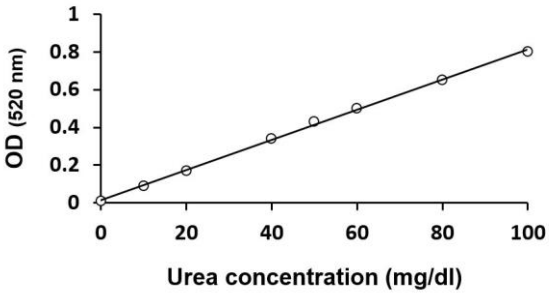
   g. Fresh human urine sample: 1501 ± 52

   h. Human BAL sample: 0.21 ± 0.03

   g. Cell culture medium: 0.15 to 2.7

EXAMPLE OF ASSAY

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. And this kit does not need serial diluted standard.



QUALITY ASSURANCE

Sensitivity

Use as little as 5 µL of samples are needed. Linear detection range 0.08 mg/dL (13 µM) to 100 mg/dL (17 mM) Urea in 96-well plate.

The minimum detectable dose (MDD) of Urea was: 0.08 mg/dL (13 µM)