Technical Bulletin

Urea Colorimetric Assay Kit II

Catalog Number MAK410

Product Description

Urea is a waste product which is produced in the liver, dissolved, and transported in the blood (in a concentration of 1.6-7.5 mM), and excreted by the kidneys. Urea plays a very important role in protein catabolism, removal of toxic ammonia from the body, and the countercurrent system, which allows for reabsorption of water and critical ions in the nephrons. Urea concentration is an important indicator for assessing the function of the kidneys and other organs.

The Urea Colorimetric Assay Kit II is based on Jung's method with a modification that delivers more robust and sensitive data. The urea is condensed with *o*-phthalaldehyde (OPA), followed by a reaction to form a colored product with strong absorbance at 505 nm. The assay is fast, sensitive, and easy to use. It can measure less than 10 μM urea in a96-well plate assay.

The kit is suitable for the measurement of urea levels in biological samples (urine, serum, plasma, etc.), tissue (liver, kidney, etc.), adherent or suspension cells (3T3 cells, Jurkat cells, etc.) and milk. It is also suitable for the analysis of liver and kidney function.

Components

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

- Urea Reagent A 12 mL
  Catalog Number MAK410A
- Urea Reagent B 12 mL
  Catalog Number MAK410B
- Urea Standard (100 mM) 100 μL
  Catalog Number MAK410C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96 well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

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 kit at 2-8 °C, protected from light. Use kit within two months of receipt.

Preparation Instructions

Urea Reagent A and Reagent B: Ready to use. Warm to room temperature prior to use.

Urea Standard (100 mM): Warm to room temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Tissue and Cell Sample Preparation

1. Homogenize tissue (10 mg) or cells (1 x 10^6 cells)with 100 µL of purified water on ice.
2. Centrifuge at 10,000 × *g* for 5 minutes.
3. Collect the supernatant.
4. Add 1-48 µL of the supernatant into the well(s) of a 96-well plate as "Sample" (S) and adjust the total volume of each well to 50 µL with purified water. For unknown samples, test different amounts of sample to ensure that readings are within the Standard Curve range.
5. For samples where background interference is suspected, prepare a paired "Spiked Sample" (SS) to correct for the sample interference. Prepare duplicate well(s) with the same volume of supernatant as in Sample (S). Add 2 µL of the 10 mM Urea Standard to each Spiked Sample (SS) well. Adjust the total volume of each well to 50 µL with purified water.

Serum and Plasma

1. Serum and plasma samples can be measured directly. Add 2-10 µL serum or plasma sample into the well(s) of a 96-well plate as "Sample" (S) and adjust the total volume of each well to 50 µL with purified water. For unknown samples, test different amounts of sample to ensure that readings are within the Standard Curve range.
2. For samples where background interference is suspected, prepare a paired "Spiked Sample" (SS) to correct for the sample interference. Prepare duplicate well(s) with the same volume of supernatant as in Sample (S). Add 2 µL of the 10 mM Urea Standard to each Spiked Sample (SS) well. Adjust the total volume of each well to 50 µL with purified water.

Urine

1. For urine samples, centrifuge samples at 10,000 × *g* for 5 minutes at room temperature.
2. Collect the supernatant.
3. Dilute the supernatant 1:50 by adding 10 µL of supernatant into 490 µL of purified water.
4. Add 2-10 µL of diluted urine sample into the well(s) of a 96-well plate as "Sample" (S) and adjust the total volume of each well to 50 µL with purified water. For unknown samples, test different amounts of sample to ensure that readings are within the Standard Curve range.
5. For samples where background interference is suspected, prepare a paired "Spiked Sample" (SS) to correct for the sample interference. Prepare duplicate well(s) with the same volume of supernatant as in Sample (S). Add 2 µL of the 10 mM Urea Standard to each Spiked Sample (SS) well. Adjust the total volume of each well to 50 µL with purified water.

Liquid samples that are not clear (for example, milk)

1. For liquid samples that are not clear, use a 10 kDa Spin Column such as Corning® Spin-X® UF concentrator to clarify the sample.
2. Use the filtrate to measure the urea content. Add 1-48 µL of the supernatant into the well(s) of a 96-well plate as "Sample" (S) and adjust the total volume of each well to 50 µL with purified water. For unknown samples, test different amounts of sample to ensure that readings are within the Standard Curve range
3. For samples where background interference is suspected, prepare a paired "Spiked Sample" (SS) to correct for the sample interference. Prepare duplicate well(s) with the same volume of supernatant as in Sample (S). Add 2 µL of the 10 mM Urea Standard to each Spiked Sample (SS) well. Adjust the total volume of each well to 50 µL with purified water.

Standard Curve Preparation

Prepare a 10 mM (10 nmol/µL) Urea standard by adding 10 µL of Urea Standard (100 mM) to 90 µL of purified water. Mix well. Prepare Urea Standards according to Table 1.

Table 1.
Preparation of Urea Standards

Well	10 mM Urea Standard	Purified Water	Urea (nmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	20
3	4 µL	46 µL	40
4	6 µL	44 µL	60
5	8 µL	42 µL	80
6	10 µL	40 µL	100

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed (Samples, Spiked Samples, and standards). For each well, prepare 200 µL of Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Volume
Urea Reagent A	100 µL
Urea Reagent B	100 µL

2. Add 200 µL of the Reaction Mix to each well containing the Standard and test samples. Mix well.

Measurement

Incubate the plate for 60 minutes at room temperature and measure absorbance at 505 nm (A_{505}).

Results

1. Subtract the 0 Standard A_{505} reading from all readings.
2. Plot the Urea Standard Curve.
3. For Known Samples with low background (no Spiked Sample used),apply the corrected Sample A_{505} readings to the Urea Standard Curve to determine the amount of Urea (X) (as nmol) in the Sample well(s).
4. For Spiked Samples, correct for sample interference by subtracting the Sample (S) A_{505} reading from the paired Spiked Sample (SS) A_{505} reading:

Urea amount (X) (in nm) in Sample for Spiked Sample =

$$\left[\frac{\text{Sample } A_{505 \text{ Corrected}}}{\text{Spiked} - \text{Sample } A_{505 \text{ Corrected}}}\right] \times 20 \text{ nmol}$$

5. Calculate Sample Urea concentration:

Urea concentration (C) (nmol/µL or mol/mL or mM) =

(X/V) × D

where:

X = Amount of Urea (nmol) from Step 3 or 4 above

V = Sample volume added into reaction well (µL)

D = Dilution factor (if applicable; D = 1 for undiluted samples)

To convert Sample Urea concentration (C) from mM (mol/mL) to mg/dL, multiply C by 6.006. Urea MW: 60.06 g/mol

To express as BUN (blood urea nitrogen):

BUN = C × 2.8011 (mg/dL)

Figure 1.
Typical Urea Standard Curve.

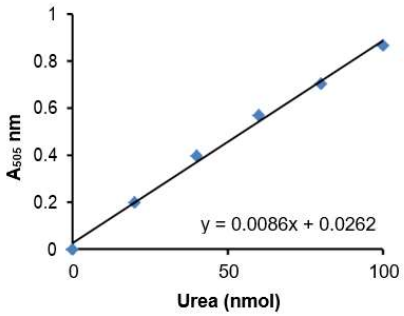
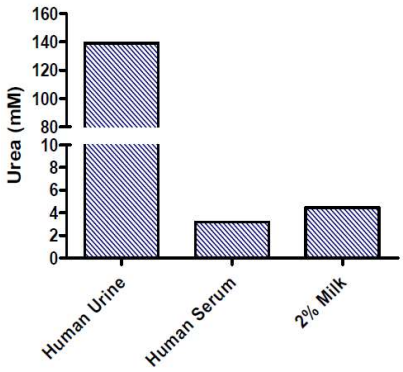


Figure 2.
Measurement of urea in human urine (5 µL of 1:50 dilution), human serum (5 µL) and 2% milk (20 µL). Assays were performed following the kit protocol.



Frequently Asked Questions:

Can urea in cell culture medium be tested?

Cells do not normally secrete urea into cell culture medium. Therefore, urea would not be expected to be detected. Any interference from FBS can be subtracted from the sample readings if needed, as long as there is enough urea in the medium volume used per well. Cell culture media has not been tested with this kit. The media, if containing phenol red, will likely change color due to the pH change during the assay and this can contribute to color.

Can the kit work on bacteria or yeast cells?

The kit has been standardized for mammalian cells only.

What is the exact volume of sample required for this assay?

There is no specific recommended volume for each sample to be used since it is completely sample concentration and quality based. Perform pilot experiments with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve.

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