



Urea Colorimetric Assay Kit II

8/13

(Catalog # K376-100; 100 assays; Store at 4°C)

I. Introduction:

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 kidney. 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 the medical clinicians to assess function of the kidney and other organs in patients. BioVision's Urea 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. This assay kit is fast, sensitive & easy to use. It can measure less than 10 μ M urea in 96-well plate assay.



II. Application:

- Measurement of urea level in various tissues/cells
- Analysis of liver and kidney function

III. Sample Type:

- Biological samples: urine, serum, plasma etc.
- Animal tissue: e.g. rat liver, kidney etc.
- Adherent or suspension cells: e.g. 3T3 cells, Jurkat cells etc.
- Milk

IV. Kit Contents:

Components	K376-100	Cap Code	Part Number
Urea Reagent A	12 ml	Blue	K376-100-1
Urea Reagent B	12 ml	Brown	K376-100-2
Urea Standard (100 mM)	100 μ l	Yellow	K376-100-3

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

VI. Storage and Handling:

Store kit at 4°C, protected from light. Warm Urea Reagent A & Reagent B to room temperature before use. Use within two months.

VII. Reagent Preparation and Storage Conditions:

Urea Standard: Dilute Urea Standard to 10 mM (10 nmol/ μ l) by adding 10 μ l of 100 mM Urea Standard to 90 μ l dH₂O. Mix well.

VIII. Urea Assay Protocol:

1. Sample Preparation: Homogenize tissue (10 mg) or cells (1×10^6) with 100 μ l dH₂O on ice. Centrifuge at 10,000 x g for 5 min. and collect the supernatant. Add 1-48 μ l supernatant into a 96-well plate and adjust the volume to 48 μ l with dH₂O. Serum and plasma samples can be measured directly. Add 2-10 μ l serum or plasma sample into a 96-well plate & adjust the final volume to 48 μ l with dH₂O. For urine samples, centrifuge samples at 10,000 x g for 5 min. at room temperature & collect the supernatant. Dilute the supernatant 50 times by adding 10 μ l of supernatant into 490 μ l dH₂O. Add 2-10 μ l diluted urine sample into a 96-well plate & adjust the volume to 48 μ l with dH₂O. For other liquid samples that are not clear such as milk, use 10 kDa spin column (cat. # 1997) to clarify and use filtrate to measure the urea content. To correct for sample interference, spike 2 μ l of the 10 mM Urea Standard into each sample well.

Note:

For unknown samples, we suggest testing several doses to ensure the reading are within the Standard Curve range.

2. Standard Curve Preparation: Add 0, 2, 4, 6, 8 and 10 μ l of the 10 mM Urea Standard into a series of wells in a 96-well plate to generate 0, 20, 40, 60, 80, and 100 nmol/well of Urea Standard. Adjust the volume to 50 μ l/well with dH₂O.

3. Reaction Mix: Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 200 μ l Reaction Mix containing:

	Reaction Mix
Urea Reagent A	100 μ l
Urea Reagent B	100 μ l

Add 200 μ l of the Reaction Mix to each well containing the Standard and test samples. Mix well.

4. Measurement: Incubate for 60 min. at room temperature. Measure absorbance (OD 505 nm).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the Urea Standard Curve. Correct for any sample interference by subtracting the sample reading from spiked sample reading. Calculate the Urea amount (X) in the sample wells:

$$\text{Urea amount in sample well, } X \text{ (nmol)} = \left(\frac{(\text{OD}_{\text{sample (corrected)}})}{(\text{OD}_{\text{sample +20(corrected)}}) - (\text{OD}_{\text{sample(corrected)}})} \right) * 20 \text{ nmol}$$

$$\text{Sample Urea concentration (C)} = X/V \times D = \text{nmol}/\mu\text{l} = \mu\text{mol}/\text{ml} \text{ or } \text{mM}$$

Where: **X** is the amount of Urea (nmol) in the sample well
V is the sample volume added into reaction well (μl)
D is the Sample Dilution Factor

To convert sample urea concentration to mg/dL, multiply C by 6.006.

Urea MW: 60.06 g/mol

To express as BUN (blood urea nitrogen):

$$\text{BUN} = C * 2.8011 \text{ (mg/dL)}$$

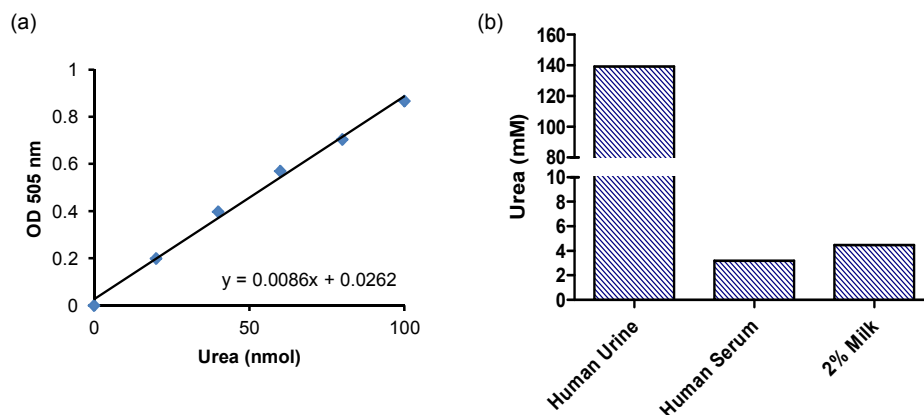


Figure: (a) Urea Standard Curve. (b) Measurement of urea in human urine (5 μl of 50 times diluted), human serum (5 μl) and 2% milk (20 μl). Assays were performed following the kit protocol.

IX. RELATED PRODUCTS:

Urea Colorimetric Assay Kit (K375)
Fumarate Colorimetric Assay Kit (K633)
Ammonia Colorimetric Assay Kit II (K470)

Aspartate Colorimetric/Fluorometric Assay kit (K552)
Ammonia Colorimetric Assay Kit (K370)

FOR RESEARCH USE ONLY! Not to be used on humans.