Urea Colorimetric Assay Kit II

(Catalog # BN00638; 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. Assay Genie'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 µM urea in 96-well plate assay.

Urea + Reagent A + Reagent B ⟶ Color Product (OD 505 nm)

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	BN00638	Cap Code	Part Number
Urea Reagent A	12 ml	Blue	BN00638-1
Urea Reagent B	12 ml	Brown	BN00638-2
Urea Standard (100 mM)	100 µl	Yellow	BN00638-3

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/µl) by adding 10 µl of 100 mM Urea Standard to 90 µl dH_2O. Mix well.

VIII. Urea Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl dH_2O on ice. Centrifuge at 10,000 x g for 5 min. and collect the supernatant. Add 1-48 µl supernatant into a 96-well plate and adjust the volume to 48 µl with dH_2O. Serum and plasma samples can be measured directly. Add 2-10 µl serum or plasma sample into a 96-well plate & adjust the final volume to 48 µl with dH_2O. 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 µl of supernatant into 490 µl dH_2O. Add 2-10 µl diluted urine sample into a 96-well plate & adjust the volume to 48 µl with dH_2O. For other liquid samples that are not clear such as milk, use 10 kDa spin column to clarify and use filtrate to measure the urea content. To correct for sample interference, spike 2 µl 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 µl 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 µl/well with dH_2O.

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

	Reaction Mix
Urea Reagent A	100 µl
Urea Reagent B	100 µl

   Add 200 µl 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:

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

Sample Urea concentration (C) = X/V x D = nmol/µl = µmol/ml or 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):

BUN = C * 2.8011 (mg/dL)

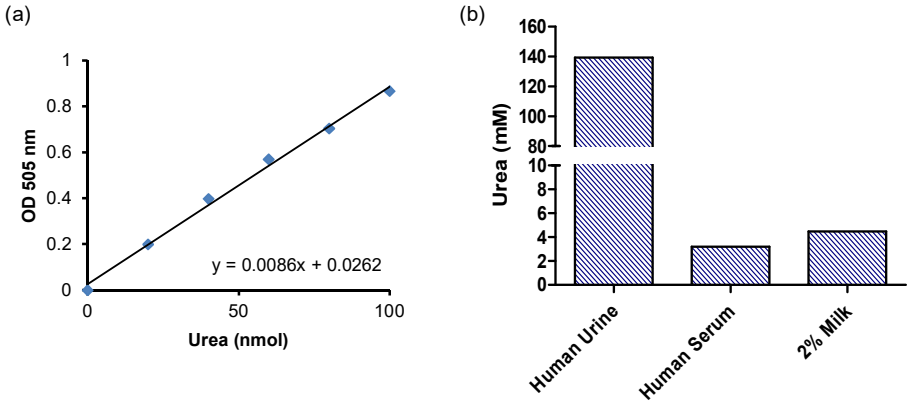

Figure: (a) Urea Standard Curve. (b) Measurement of urea in human urine (5 µl of 50 times diluted), human serum (5 µl) and 2% milk (20 µl). Assays were performed following the kit protocol.

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