NOTE: Revision to
"Preparation of Reagents"

Product Manual

Urea Assay Kit

Catalog Number

STA-382 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Urea, or carbamide, is the end product of protein nitrogen metabolism and is the primary vehicle for removing toxic ammonia from the body. Urea is synthesized in the liver from the ammonia produced from the catabolism of amino acids via the hepatic urea cycle. The conversion from ammonia to urea is regulated by N-acetylglutamate, which activates carbamoyl phosphate synthetase in the urea cycle. Urea is transported in the blood to the kidneys where it is excreted in the urine. In addition to its role as a carrier of waste nitrogen, urea also has a role in the countercurrent exchange system of the nephrons in which water and ions are re-absorbed from excreted urine. It is freely filtered by the glomeruli and partially passively resorbed as filtrate transverses the renal tubules. Urea reabsorption is inversely proportional to urine flow rate. Consequently, urea concentration depends upon protein intake, protein catabolism, and kidney function.

Urea quantitation is one of the most widely applied tests for kidney function evaluation. The analysis of urea in serum, plasma and urine is an important clinical test for renal disease and dysfunction. The test is frequently tested in conjunction with creatinine determination for diagnosis of pre-renal, renal, and post renal uremia. Toxic urea levels are associated with renal, liver, or other system dysfunction. Pre-renal uremia relates to water depletion, increased protein catabolism, infection, hypovolemia, or cardiac decomposition. Glomerulonephritis, tubular necrosis, nephrosclerosis, chronic nephritis, and polycystic kidney are examples of renal uremia, while post renal uremia is predominantly urinary tract obstructions or leakage. Increased urea levels can also be linked to other disease states such as liver disease, diabetes, and congestive heart failure. High plasma urea levels are known as Azotemia. Decreased urea levels are associated with acute hepatic insufficiency or excess parenteral fluid therapy.

Cell Biolabs' Urea Assay Kit is based on the Berthelot reaction. Urea is first degraded into ammonia and carbon dioxide, which further reacts with an alkaline developer to produce a blue-green colored product that can be measured with a standard spectrophotometric plate reader at an optical density between 580-630 nm. Each kit provides sufficient reagents to perform up to 200 assays, including blanks, urea standards and unknown samples and controls.

Assay Principle

Cell Biolabs' Urea Assay Kit measures urea levels within urine, serum, plasma, cell lysates, or tissue homogenates. Samples are compared to a known concentration of urea standard within a 96-well microtiter plate format. Samples and standards are incubated for 10 minutes with the enzyme urease, which hydrolyzes urea to ammonia and CO_2. The ammonia reacts further with a chromogen in alkali solution to produce a blue-green colored product. After 30 minutes, the plate is read with a standard 96-well spectrophotometric microplate reader at an optical density between 580 nm and 630 nm (Figure 2). Higher OD values correlate with high urea concentrations. Sample urea concentrations are determined by comparison with the known urea standards. The standard curve is linear up to 50 mg/dL urea.

Related Products

1. STA-375: Uric Acid/Uricase Assay Kit
2. STA-378: Creatinine Assay Kit

Kit Components

Box 1 (shipped at room temperature)

1. Urea Standard (Part No. 238201): One 250 µL tube of a 1000 mg/dL solution.
2. Ammonia Reagent (Part No. 238202): One 20 mL amber bottle.
3. Developing Reagent (Part No. 238203): One 20 mL bottle.
4. 10X Assay Buffer (Part No. 238205): One 10 mL bottle.

Box 2 (shipped on blue ice packs)

1. Urease (Part No. 238204): One 200 mg amber tube of powder.

Materials Not Supplied

1. Standard 96-well microtiter plates for use in microplate reader
2. Deionized water
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoirs
6. 37ºC incubator
7. Spectrophotometric microplate reader capable of reading 580-630 nm

Storage

Upon receipt, prepare aliquots and store the Urea Standard and Urease at -20ºC. Store the remaining kit components at 4ºC.

Preparation of Reagents

- 1X Assay Buffer: Dilute the Assay Buffer 1:10 with deionized water. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC up to six months.
- Urease/Ammonia Reagent*: Immediately prior to use, reconstitute the Urease enzyme at 4 mg/mL in the Ammonia Reagent solution and mix thoroughly until dissolved (e.g. for a 10 mL solution or 100 assays, add 40 mg of Urease to 10 mL Ammonia Reagent). Prepare only enough for immediate use. Do not store the Urease/Ammonia Reagent solution.

  **Note: Some sample types may have high levels of ammonia, which may cause high background values. For these samples types we recommend two assays per sample for testing: one well treated with the Urease (+U) added to the Ammonia Reagent, and one with Urease omitted from the Ammonia Reagent (-U). The urea concentration is calculated from the difference in OD readings between the two assay values.*

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. All samples should be assayed immediately or stored for up to 2 months at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering chromogens. High levels of interfering substances may cause variations in results. Samples may be diluted in 1X Assay Buffer or deionized water as necessary before testing. Run proper controls as necessary. Always run a standard curve with samples.

- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C. Perform dilutions in deionized water.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood sample and add to a blood collection tube containing Heparin as the anticoagulant. Centrifuge at 3,000 rpm for 10-15 minutes at 4ºC. Remove the upper yellow plasma supernatant layer without disturbing the white buffy coat (leukocytes). Samples should be tested immediately or frozen at -80°C. Perform dilutions in deionized water.
- Urine: Urine samples with visible particulates should be centrifuged or filtered prior to testing. A minimum 1:20 dilution of urine samples into deionized water is recommended to remove matrix interference and achieve optimal assay results. Diluted samples should be used within 2 hours upon preparation.
- Tissue or Lysates: Homogenize 20 mg of tissue or $2x10^6$ cells in cold 1X Assay Buffer. Centrifuge at 14000 x g for 10 min to remove insoluble material. Collect the supernatant. Samples can be tested directly or diluted with 1X Assay Buffer. Test samples immediately or store at -80°C.

  Notes:

  - *Buffers containing MES, HEPES, CHES, EDTA, fluoride, 2-mercaptoethanol, acetohydroxamate, 1,4-benzoquinone, or phosphoramidate are not recommended because they can inhibit urease activity.*
  - *Do not use ammonium or potassium salts or fluoride as anticoagulants. Citrate, sodium heparin or oxalate can be used. All samples must be free of heavy metals.*
  - *Hemoglobin (>200 mg/dL), Bilirubin (>20 mg/dL), and Triglycerides (>800 mg/dL) may interfere with the assay. Use controls accordingly.*
  - *Drug interferences are possible (see Young, D.S., et. al).*

Preparation of Urea Standard Curve

1. Prepare fresh urea standards by diluting in deionized water. First, dilute the stock Urea Standard 1000 mg/dL solution 1:20 in distilled or deionized water for a 50 mg/dL solution. (e.g. Add 25 µL of the stock 1000 mg/dL standard to 475 µL of deionized water).
2. Use this 50 mg/dL solution to prepare a series of the remaining urea standards according to Table 1 below.

Tubes	50 mg/dL Urea Standard (µL)	Deionized Water (µL)	Resulting Urea Concentration (mg/dL)
1	500	0	50
2	250 of Tube #1	250	25
3	250 of Tube #2	250	12.5
4	250 of Tube #3	250	6.25
5	250 of Tube #4	250	3.13
6	250 of Tube #5	250	1.56
7	250 of Tube #6	250	0.78
8	0	500	0.0

Table 1. Preparation of Urea Standards.

Note: Do not store diluted urea standard solutions.

Assay Protocol

Each urea standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Note: Samples with high concentration of ammonia require two paired wells, one to be treated with the Urease/Ammonia Reagent mixture (+U), and one with the Ammonia Reagent without Urease (-U).This will allow for the determination of background ammonia levels within the sample.

1. Add 10 µL of the diluted urea standards or samples to the 96-well microtiter plate wells. Remember to provide two sample paired wells if testing for ammonia background.
2. Add 100 µL of the Urease/Ammonia Reagent (+U) mixture (see Preparation of Reagents section) to each standard and sample well using either a multichannel pipette or a plate reader liquid handling system. Mix thoroughly and carefully to avoid foaming in the well.

   Note: For the high ammonia-containing samples requiring paired wells, add 100 µL of the Ammonia Reagent (-U) to one of the paired wells for each sample.
3. Incubate 10 minutes at 37ºC.
4. Add 100 µL of the Developing Reagent to each well using either a multichannel pipette or a plate reader liquid handling system. Mix the solution thoroughly and carefully so as not to create foaming in the well.
5. Incubate 30 minutes at 37ºC.
6. Read the plate at 580-630 nm and record data.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.

2. Subtract the average zero standard value from itself and all standard and sample values. This is the background correction.

3. Graph the standard curve (see Figure 2).

4. For samples with two paired wells (with and without urease), subtract the sample well absorbance values without urease (A_{-U}) from the urease-treated sample well absorbance values (A_{+U}) to obtain the absorbance difference. The (A_{-U}) sample value represents the ammonia background concentration within the sample, while the (A_{+U}) sample value is the combined urea and ammonia background concentration within the sample. The absorbance difference (ΔA) is due to the urea concentration:

$$(\Delta A) = (A_{+U}) - (A_{-U})$$

5. Compare the absorbance values of each sample to the standard curve to determine and extrapolate the quantity of urea present in the sample. Only use values within the range of the standard curve.

Example of Results

The following figures demonstrate typical Urea Assay results. One should use the data below for reference only. This data should not be used to interpret actual sample results.

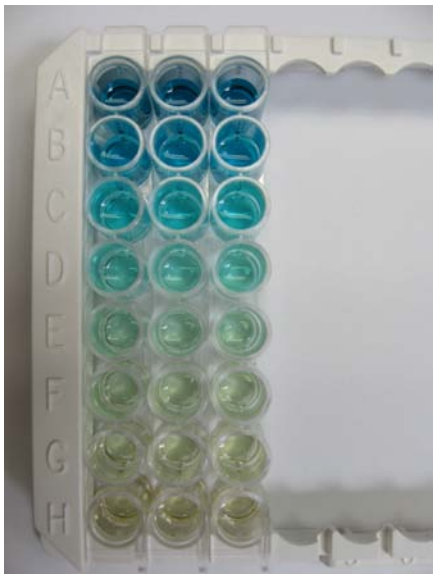

Figure 1: Urea Assay Standard Curve. Typical color visualization of standards generated using the Cell Biolabs Urea Assay Kit.

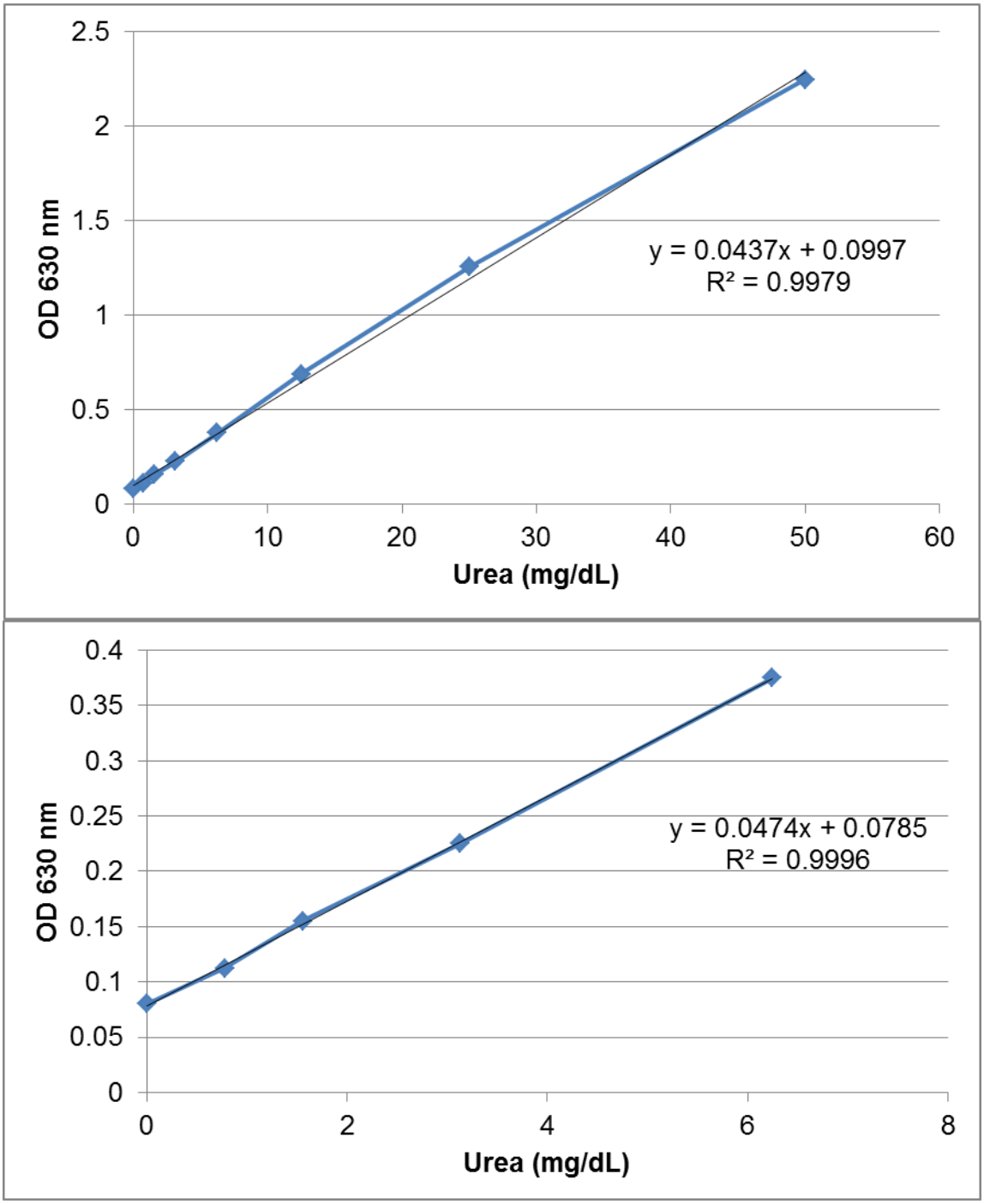

Figure 2: Urea Assay Standard Curve.

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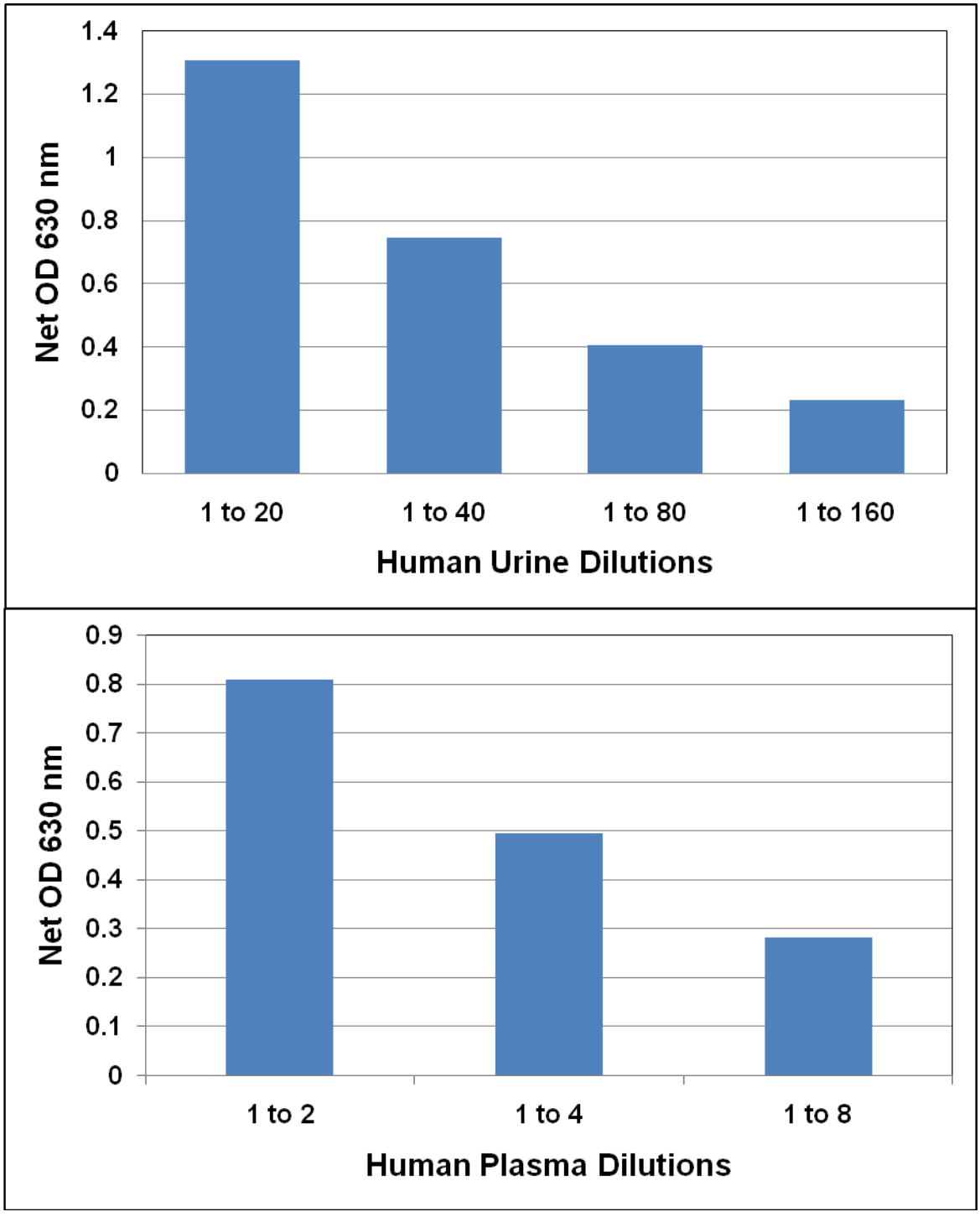

Figure 3: Urine and Plasma Samples. Human urine and plasma samples were tested with the Urea Assay Kit.

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Recent Product Citations

1. Kurowski, M., et al. (2017). A similar pro/anti-inflammatory cytokine balance is present in the airways of competitive athletes and non-exercising asthmatics. *Adv Med Sci*. **63**(1):79-86. doi: 10.1016/j.advms.2017.07.004.
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Warranty

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