Datasheet for ABIN7041642

Urea Assay Kit

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Overview

Quantity:	200 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	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 CO2. 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 . 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.
Sample Type:	Cell Lysate, Serum, Plasma, Urine
Characteristics:	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 192 assays, including blanks, urea standards and unknown samples.
Components:	1. Urea Standard : One 250 µL tube of a 1000 mg/dL solution. 2. Ammonia Reagent : One 20 mL amber bottle. 3. Developing Reagent : One 20 mL bottle. 4. 10X Assay Buffer : One 10 mL bottle. Box 2 (shipped on blue ice packs)

Product Details

Material not included:	1. Standard 96-well microtiter plates for use in microplate reader 2. 1X PBS and 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 reservoir 6. Orbital shaker 7. 37 °C incubator 8. Spectrophotometric microplate reader capable of reading 580-630 nm

Target Details

Background:	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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.

Application Details

Comment:	• Sample urea concentrations are determined by comparison with the known urea standards,the standard is linear up to 200 mg/dL urea • Measures urea levels within urine, serum, plasma, cell lysates, or tissue homogenates • Provides sufficient reagents to perform up to 192 assays, including blanks, urea standards and unknown samples
Reagent Preparation:	• 1X Assay Buffer: Dilute the Assay Buffer 1:10 with deionized water. Mix to homogeneity. Store the 1X Assay Buffer at 4 °C 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.
Sample Preparation:	Samples should be stored at -80 °C prior to performing the assay. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering chromogens. 3 • Serum or Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin and centrifuge at 4 °C for 10 minutes. Remove the plasma and aliquot samples for testing. A minimum 1:10 dilution is recommended. 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 2x106 cells in 1X Assay Buffer. Centrifuge at 14000 x g for 10 min to remove insoluble material. Samples can be tested directly or diluted with 1X Assay Buffer. 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 ammonia and 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).
Assay Procedure:	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. 1. Add 10 µL of the diluted urea standards or samples to the 96-well microtiter plate wells. 2. Prior to use, reconstitute the Urease enzyme at 4 mg/mL in the Ammonia Reagent solution and mix thoroughly until dissolved (eg. For a 10 mL solution or 100 assays, add 40 mg of Urease to 10 mL Ammonia Reagent).

Application Details

3. Add 100 µL of the Urease/Ammonia Reagent mixture to each well using either a multichannel pipette or a plate reader liquid handling system. Mix thoroughly and carefully so as not to create foaming in the well.
4. Incubate 10 minutes at 37 °C.
5. 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.
6. Incubate 30 minutes at 37 °C.
7. Read the plate at 580-630 nm and record data.

Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, prepare aliquots and store the Urea Standard and Urease at -20°C. Store the remaining kit components at 4°C.

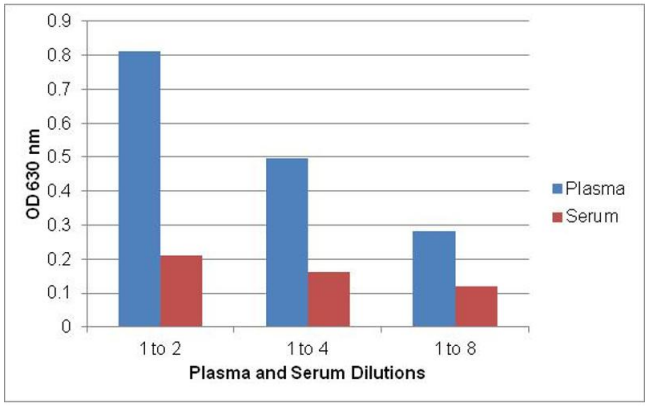
Publications

Product cited in:

Trapani, Tricarico, Mele, Maqoud, Mandracchia, Vitale, Capriati, Trapani, Dimiccoli, Tolomeo, Scilimati: "A novel injectable formulation of 6-fluoro-l-DOPA imaging agent for diagnosis of neuroendocrine tumors and Parkinson's disease." in: **International journal of pharmaceutics**, Vol. 519, Issue 1-2, pp. 304-313, (2017) (PubMed).

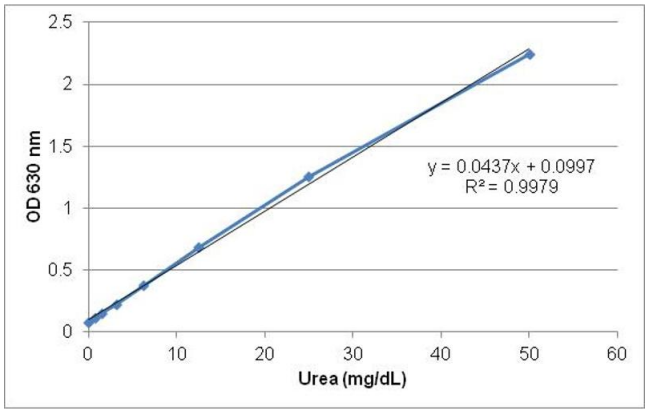
Kang, Lee, Park, Yi, Lee, So, Song, Kang: "Chemically induced hepatotoxicity in human stem cell-induced hepatocytes compared with primary hepatocytes and HepG2." in: **Cell biology and toxicology**, Vol. 32, Issue 5, pp. 403-17, (2016) (PubMed).

Bruinsma, Yeh, Ozer, Martins, Farmer, Wu, Saeidi, Op den Dries, Berendsen, Smith, Markmann, Porte, Yarmush, Uygun, Izamis: "Subnormothermic machine perfusion for ex vivo preservation and recovery of the human liver for transplantation." in: **American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons**, Vol. 14, Issue 6, pp. 1400-9, (2014) (PubMed).



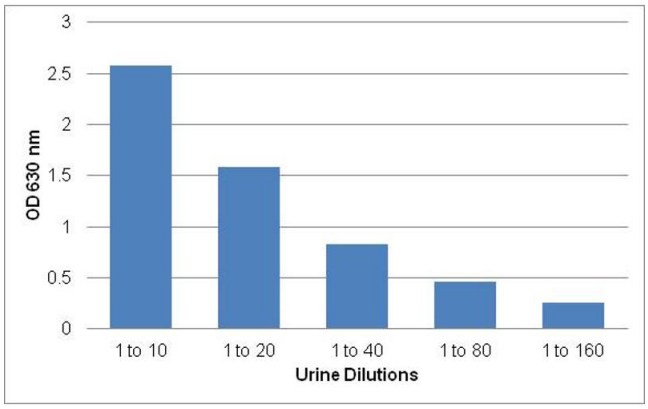
ELISA

Image 1. Human Plasma and Serum Sample Dilutions Tested with the Urea Assay Kit.



ELISA

Image 2. Urea Assay Standard Curve.



ELISA

Image 3. Human Urine Sample Dilutions Tested with the Urea Assay Kit.

