Product Manual

Urinary Creatinine Assay Kit

Catalog Number

STA-378 192 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Creatinine (2-Amino-1-methyl-2-imidazolin-4-one) is a metabolite formed from creatine and phosphocreatine, which are found almost exclusively in skeletal muscle. Phosphocreatine (p-creatine) is a molecule that stores high-energy phosphate, which can be used by tissues for ATP production. The majority of creatine is found in muscle, as well as the heart, brain, testes, and photoreceptors. Creatine can come from the diet or can be synthesized from the amino acids arginine, glycine, and methionine. Although this occurs mainly in the liver and kidneys, other organ systems may be involved.

Creatine and p-creatine are converted non-enzymatically to creatinine, which enters the blood and is excreted by the kidneys via glomerular filtration (Figure 1). This conversion appears irreversible *in vivo,* while i*n vitro* it is favored by high temperature and lower pH. Creatinine forms spontaneously from p-creatine and usually forms at a constant rate. Intra-individual variation of creatinine levels is less than 15% daily, which makes it a useful marker for normalizing levels of other molecules found in the urine. Creatinine production is proportional to muscle mass and is usually consistent from one day to the next; however, changes can occur over a longer period if there are changes in muscle mass. Altered creatinine levels can be an indicator of kidney dysfunction or other medical conditions that result in lower renal blood flow such as in diabetes or cardiovascular disease.

The analysis of creatinine in serum and urine is an important clinical test for renal disease and dysfunction. Creatinine is removed from plasma by the glomerulus and then excreted in the urine without any appreciable resorption by the tubules. This "creatinine clearance" from the body is used to measure glomerular filtration rates. Serum creatinine concentration is related to muscle mass. Increased serum creatinine is associated with decrease in glomerular filtration rate (GFR); however, serum creatinine levels do not rise until renal function has decreased by at least 50%. Independent of diet, serum creatinine concentration depends upon its excretion rate from the kidneys.

Cell Biolabs' Urinary Creatinine Assay Kit is based on the Jaffe reaction, which is a reaction between creatinine and alkaline picrate to produce an orange-red color complex that can be measured with a standard spectrophotometric plate reader at an optical density of 490 nm. Each kit provides sufficient reagents to perform up to 192 assays, including blanks, creatinine standards and unknown urine samples. High concentrations of ascorbic acid, uric acid, glucose, ketones and cephalosporin antibiotics may interfere with the assay causing falsely high values.

Assay Principle

Cell Biolabs' Urinary Creatinine Assay Kit measures creatinine levels in urine. Samples are compared to a known concentration of creatinine standard within a 96-well microtiter plate format. Samples and standards are incubated for 30 minutes with a reaction reagent which changes color from yellow to bright orange upon reacting with creatinine, forming the creatinine-picrate complex. The plate is read with a standard 96-well spectrophotometric microplate reader at 490 nm (Figure 1). Higher OD values correlate with high creatinine concentrations. Sample creatinine concentrations are determined by comparison with the known creatinine standards. Interference from non-specific chromogens can be measured by adding the creatinine quencher, which destroys the creatinine-picrate complex, thus eliminating all absorbance from creatinine. The remaining absorbance is from the non-specific chromogens, which can be subtracted from the overall values.

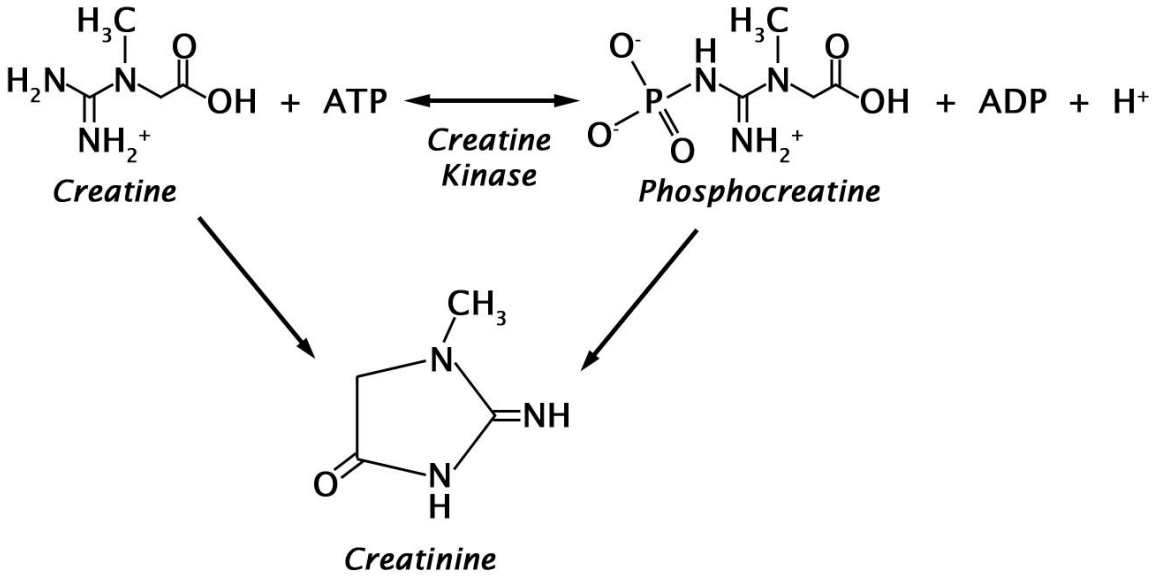

Figure 1: Creatinine Synthesis Chemistry.

Related Products

1. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
2. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
3. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
4. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
5. STA-345: OxiSelect™ ORAC Activity Assay
6. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)

Kit Components

1. 96-well Microtiter Plate (Part No. 231001): Two 96-well strip plates.
2. Creatinine Standard (Part No. 237801): One 0.5 mL vial of a 100 mg/dL Creatinine solution.
3. Creatinine Reaction Buffer (Part No. 237803): One 30 mL bottle.
4. Acid Solution (Part No. 237804): One 10 mL bottle.

Materials Not Supplied

1. 1X PBS and deionized water
2. 100 mM HCl
3. Glacial Acetic Acid
4. Sonicator or homogenizer for sample preparations
5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
6. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
7. Spectrophotometric microplate reader capable of reading 490 nm

Storage

Upon receipt store the Creatinine Standard and Acid Solution at 4ºC. Store all remaining kit components at room temperature.

Preparation of Reagents

- Creatinine Reaction Reagent: Combine 3 parts of the Creatinine Reaction Buffer with 1 part Acid Solution (e.g. for 100 assays, combine 15 mL of Creatinine Reaction Buffer with 5 mL of Acid Solution). Mix thoroughly. Store this Creatinine Reaction Reagent at room temperature for up to one week.

Preparation of Urine Samples

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.

Undiluted urine samples should be stored at -80ºC prior to performing the assay. 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 assayed within 2 hours of preparation.

Note: High concentrations of ascorbic acid, uric acid, glucose, ketones and cephalosporin antibiotics may interfere with the assay causing falsely high values. Do not report the result from specimens with suspected interfering chromogens.

Preparation of Creatinine Standard Curve

1. Prepare fresh creatinine standards by diluting in 100 mM HCl. First, dilute the stock Creatinine Standard 100 mg/dl solution 1:5 in 100 mM HCl for a 20 mg/dl solution. (e.g. add 100 µL of the stock 100 mg/dl standard to 400 µL of 100 mM HCl).
2. Use this 20 mg/dl solution to prepare a series of the remaining creatinine standards according to Table 1 below.

Tubes	20 mg/dl Creatinine Standard (µL)	100 mM HCl (µL)	Resulting Creatinine Concentration (mg/dl)
1	500	0	20
2	250 of Tube #1	250	10
3	250 of Tube #2	250	5
4	250 of Tube #3	250	2.5
5	250 of Tube #4	250	1.25
6	250 of Tube #5	250	0.625
7	250 of Tube #6	250	0.313
8	250 of Tube #7	250	0.156
9	250 of Tube #8	250	0.078
10	0	500	0.0

Table 1. Preparation of Creatinine Standards.

Note: Do not store diluted creatinine standard solutions.

Assay Protocol

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

1. Add 50 µL of the diluted creatinine standards or urine samples to the 96-well microtiter plate.
2. Add 200 µL of the prepared Creatinine Reaction Reagent 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.
3. Incubate 30 minutes on an orbital shaker at room temperature.
4. Read the plate at 490 nm and record data. These are the initial absorbance values.
5. Add 50 µL of Glacial Acetic Acid to samples and standard wells. Mix thoroughly. Incubate for 5-10 minutes on an orbital shaker. Reread the plate again at 490 nm and record data. These are the final absorbance values.

   Note: The difference in absorbance before and after adding the Glacial Acetic Acid is due to creatinine-picrate interactions. The Glacial Acetic Acid will permanently destroy the creatinine-picrate complex and any associated absorbance values. Ensure that data for all standards and samples are recorded prior to adding the Glacial Acetic Acid to wells.

Example of Results

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

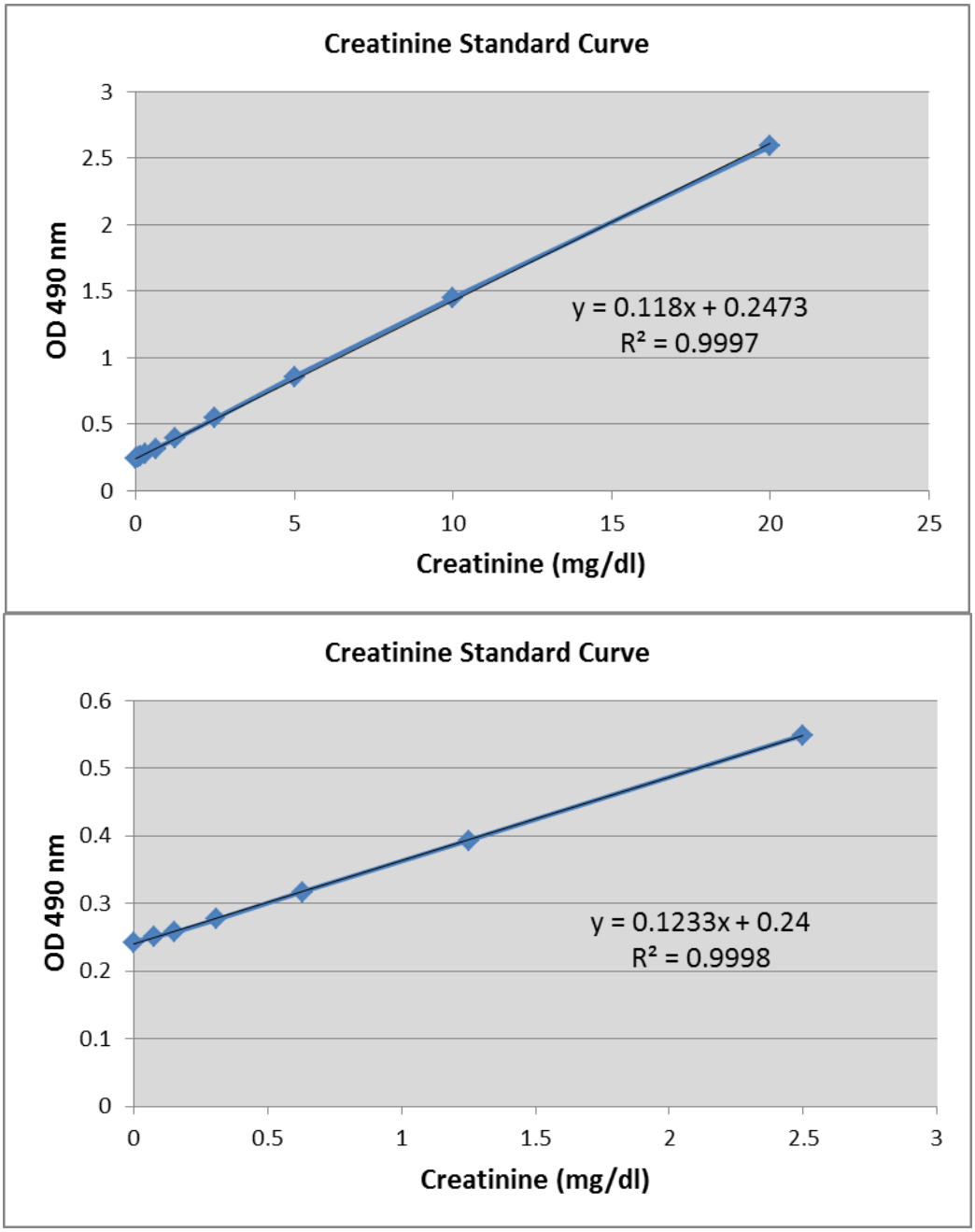

Figure 2: Creatinine Assay Standard Curve.

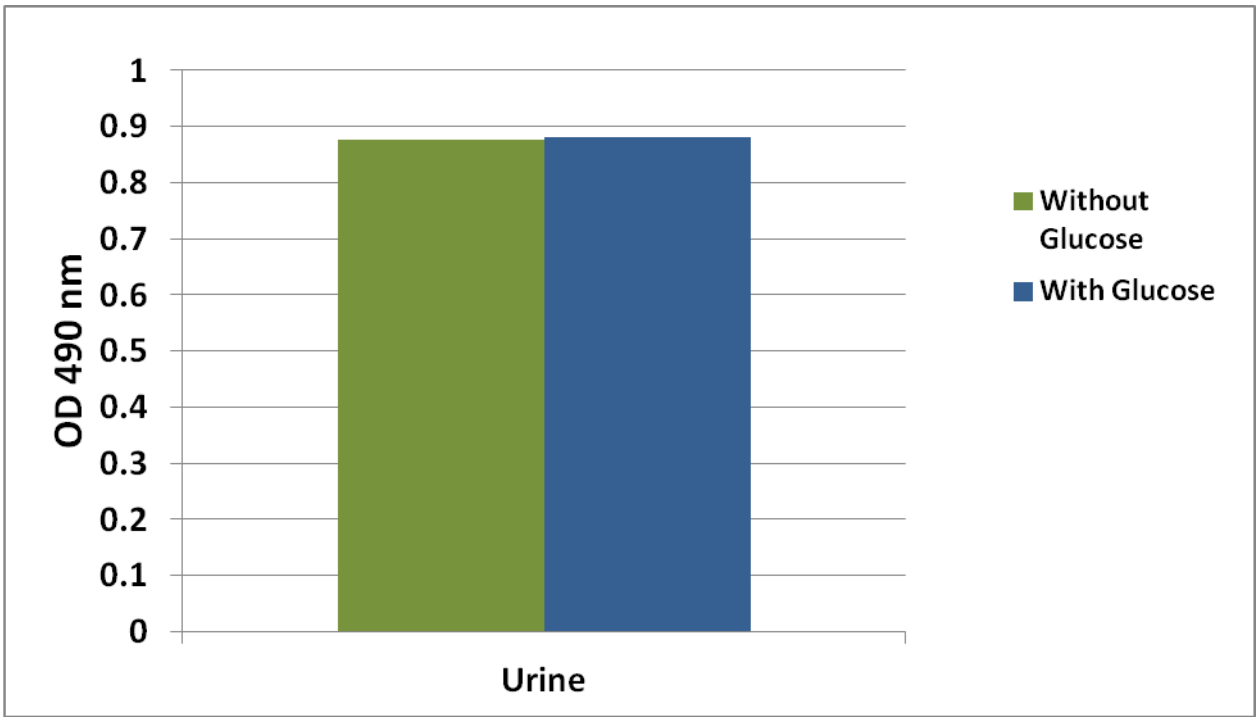

Figure 3: Specificity of the Creatinine Assay Kit in the presence of glucose. Human urine was diluted 1:20 in 0.1 N HCl alone or 0.1 N HCl plus 2000 mg/dl glucose and tested with the Creatinine Assay Kit. The results show no significant change in creatinine absorbance in the presence of high glucose concentrations.

Calculation of Results

1. Calculate the initial average absorbance (A_i) values for each standard and sample.
2. Calculate the final average absorbance (A_f) values for each standard and sample.
3. Subtract the final absorbance from the initial absorbance for the corrected absorbance (A_c).

$$(A_c) = (A_i) - (A_f)$$

4. Plot the absorbance for the creatinine standards versus the concentration of the creatinine standards to determine the best curve. Data can be linearized with log paper or regression analysis software applications.
5. Use the equation obtained from the linear regression of the standard curve to calculate the creatinine concentration of samples. Remember to account for all dilution factors. See Figure 2 for a typical standard curve equation.

$$\text{Creatinine (mg/dL)} = \left[\frac{(A_c) - \text{y intercept}}{\text{slope}} \right] \times \text{sample dilution}$$

Note: Multiply the creatinine concentration in mg/dl by 88.4 to convert the values into µmol/L (SI Unit conversion).

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Recent Product Citations

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