Datasheet for ABIN2345043
Urinary Creatinine Assay Kit

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Overview

Quantity:	192 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	Urinary Creatinine Assay Kit measures creatinine levels in urine.
Sample Type:	Urine
Sensitivity:	300 μg/dl
Characteristics:	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.
Components:	1. 96-well Microtiter Plate : Two 96-well strip plates. 2. Creatinine Standard : One 0.5 mL vial of a 100 mg/dL Creatinine solution. 3. Creatinine Reaction Buffer : One 30 mL bottle. 4. Acid Solution : One 10 mL bottle.
Material not included:	1. 1X PBS and deionized water 2. Sonicator or homogenizer for sample preparations 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. Spectrophotometric microplate reader capable of reading 490 nm

Target Details

Background:	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 in 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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Measures creatinine levels in urine samples • Detection sensitivity limit of 300 µg/dl • Creatinine standard included
Protocol:	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 . 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.
Reagent Preparation:	• 1X Assay Diluent: Dilute the Assay Diluent 1:10 with deionized water. Mix to homogeneity. Store the 1X Assay Diluent at 4 °C up to six months. • Creatinine Reaction Reagent: Combine 3 parts of the Creatinine Reaction Buffer with 1 part Picric Acid Solution (eg. For 100 assays, combine 15 mL of Creatinine Reaction Buffer with 5 mL of Picric Acid). Mix thoroughly. Store this Creatinine Reaction Reagent at room temperature for up to one week. Note: Picric Acid is a strong oxidizing agent and is toxic. Exercise caution and use safety measures while handling. Avoid contact with eyes, skin, and clothing. If contact is made, flush with copious amounts of water. Do not let the solution dry or crystalize as the dry powder is explosive. Use plenty of water to remove spilled picric acid. 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 -80 °C 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.
Assay Procedure:	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 the Creatinine Quencher 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 quencher is due to creatinine- picrate interactions. The Creatinine Quencher 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 quencher to wells.

Application Details

Calculation of Results:	1. Calculate the initial average absorbance (Ai) values for each standard and sample. 2. Calculate the final average absorbance (Af) values for each standard and sample. 3. Subtract the final absorbance from the initial absorbance for the corrected absorbance (Ac). (Ac) = (Ai) - (Af) 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. (Ac) - y intercept Creatinine (mg/dL) = x sample dilution slope Note: Multiply the creatinine concentration in mg/dl by 88.4 to convert the values into µmol/L (SI Unit conversion). 7
Restrictions:	For Research Use only

Handling

Storage:	RT/4 °C
Storage Comment:	Upon receipt store the Creatinine Standard and Picric Acid Solution at 4°C. Store all remaining kit components at room temperature.

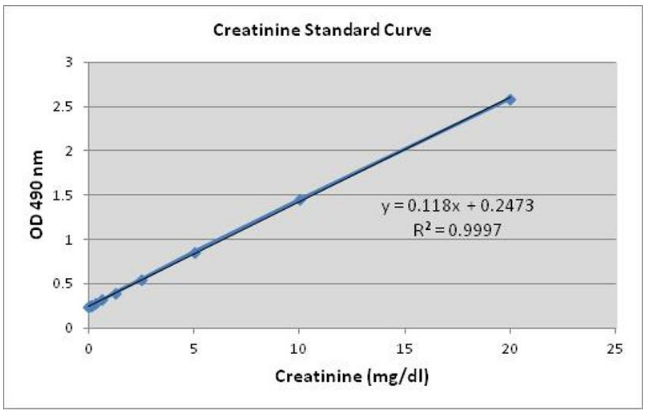
Publications

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). Ismail, deKemp, Hadizad, Mackasey, Beanlands, DaSilva: "Decreased renal AT1 receptor binding in rats after subtotal nephrectomy: PET study with [(18)F]FPyKYNE-losartan." in: **EJNMMI research**, Vol. 6, Issue 1, pp. 55, (2016) (PubMed). Bone, Gai, Magrioti, Kokotou, Ali, Lei, Tse, Kokotos, Ramanadham: "Inhibition of Ca2+-independent phospholipase A2? (iPLA2?) ameliorates islet infiltration and incidence of diabetes in NOD mice." in: **Diabetes**, Vol. 64, Issue 2, pp. 541-54, (2015) (PubMed). Zis, McHugh, McQuillin, Praticò, Dickinson, Shende, Walker, Strydom: "Memory decline in Down syndrome and its relationship to iPF2alpha, a urinary marker of oxidative stress." in: **PLoS ONE**, Vol. 9, Issue 6, pp. e97709, (2014) (PubMed).

Publications

Day, Cavaglieri, Feliers: "Apelin retards the progression of diabetic nephropathy." in: **American journal of physiology. Renal physiology**, Vol. 304, Issue 6, pp. F788-800, (2013) (PubMed).

Images



ELISA

Image 1. Creatinine Assay Standard Curve.