Urine Creatinine (UCr)

Colorimetric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44110-96

www.zellx.de

Sample Types Validated for:

Human, Monkey, Dog, and Rat Urine

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

Table of Contents

Please read this insert completely prior to using the product.

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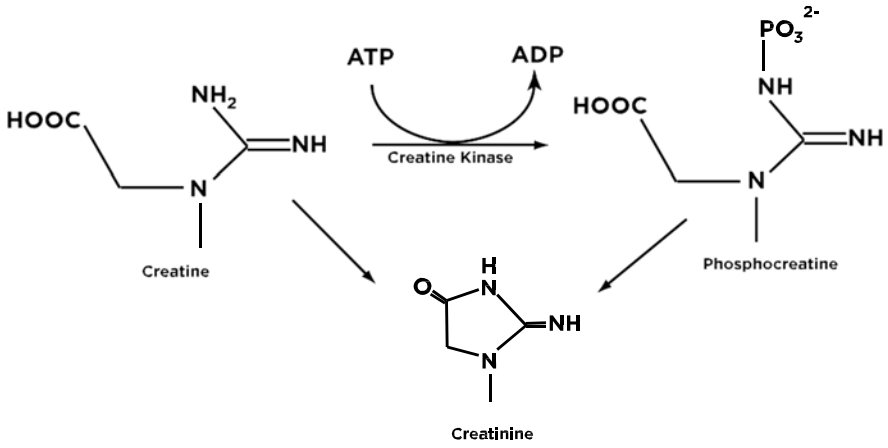
Introduction

Background

Creatinine (2-amino-1-methyl-5H-imidazol-4-one) is a metabolite of phosphocreatine (p-creatine) mainly in skeletal muscle tissues. P-creatine is the phosphorylated creatine which serves as a store for high-energy phosphate to be utilized for the production of ATP. Creatine either comes from the diet or synthesized from the amino acids arginine, glycine, and methionine. Creatine and p-creatine are converted non-enzymatically to the metabolite creatinine, which diffuses into blood and is excreted by kidneys. In vivo, this conversion appears to be irreversible and in vitro it is favored by higher temperatures and lower pH. Under normal conditions, its formation occurs at a rate that is relatively constant. Altered creatinine levels may be associated with conditions that result in decreased renal blood flow such as diabetes and cardiovascular disease.



Assay principle

The ZellX® Urine Creatinine Kit is designed to quantitatively measure creatinine present in urine samples. A creatinine standard, calibrated to the standard of NIST (National Institute of Standards and Technology), is provided to generate a standard curve for the assay, and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate. An assay diluent is added to all standards, controls and samples. The color generating reaction is initiated with the ZellX® Creatinine Reagent, which is pipetted into each well. The Jaffe reaction used in this kit has been modified to read creatinine levels in urine.

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General information

Materials supplied in the Kit

Component	*Quantity*
Creatinine Standard (1000 mg/L)	200 µL
Creatinine Reagent	10 mL
Clear Half Area 96 Well Plate	1 plate

Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

Materials required but not supplied

Double distilled water (ddH_2O)

Microplate/ELISA reader capable of reading optical absorption at 490 nm

Precision pipettes, multichannel pipette and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Creatinine Reagent contains hazardous chemicals. It contains a solution of basic picric acid in a stabilizing solution. The contact with skin or eyes must be avoided. Picric acid is an irritant and, if dried, potentially explosive. Avoid contact with metals and use large volumes of water during disposal. Take appropriate precautions when handling these reagents.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed. The reading of Microplate/ELISA reader must be set as at the appropriate wavelength of determining the experiment result.

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- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- This assay has been validated for human, rat, dog and monkey urine samples. **Mouse urine samples are not compatible with this assay**.

Assay protocol

Sample preparation

Samples must be diluted in ddH_2O. Dilutions should be made to ensure that creatinine levels for samples fall within the standard curve range.

As the creatinine level of Rhesus monkey urine is too low and should be diluted 1:2 in ddH_2O (by taking one part of urine and adding to one part of ddH_2O). All other urine samples must be diluted 1:20 with ddH_2O (by taking one part of urine and adding to 19 parts of ddH_2O).

All samples must be used within 2 hours of dilution.

Standard preparation

- Prepare a 1:5 dilution of Creatinine Standard with ddH_2O (mix 100 µL of standard with 400 µL of ddH_2O), and label as the Standard No.7 (200 mg/L).
- Apply series of other dilutions as described in the table.
- The ddH_2O is used as the 0 mU/mL standard.

No.	*Concentration*	*Material needed*
Standard No.7	200 mg/L	100 µL Creatinine Standard + 400 µL ddH_2O
Standard No.6	100 mg/L	200 µL Standard No.7 + 200 µL ddH_2O
Standard No.5	50 mg/L	200 µL Standard No.6 + 200 µL ddH_2O
Standard No.4	25 mg/L	200 µL Standard No.5 + 200 µL ddH_2O
Standard No.3	12.5 mg/L	200 µL Standard No.4 + 200 µL ddH_2O
Standard No.2	6.25 mg/L	200 µL Standard No.3 + 200 µL ddH_2O
Standard No.1	3.125 mg/L	200 µL Standard No.2 + 200 µL ddH_2O
Standard No.0	0 mg/L	200 µL ddH_2O

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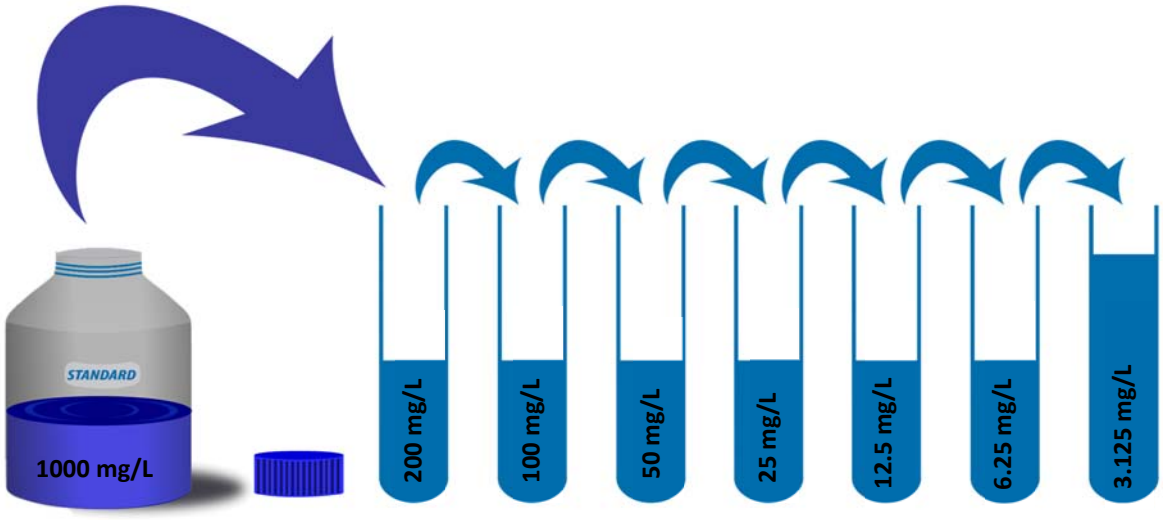

All standard must be used within 2 hours of preparation

Assay Procedure

1. Pipet 50 µL of either samples or standards into duplicate wells in the plate.
2. Pipet 50 µL of ddH_2O as the Zero standard.
3. Add 100 µL of the Creatinine Reagent to each well using a multichannel pipet.
4. Gently tap the side of the plate and mix well.
5. Incubate at room temperature for 30 minutes.
6. Read the optical density at 490 nm.

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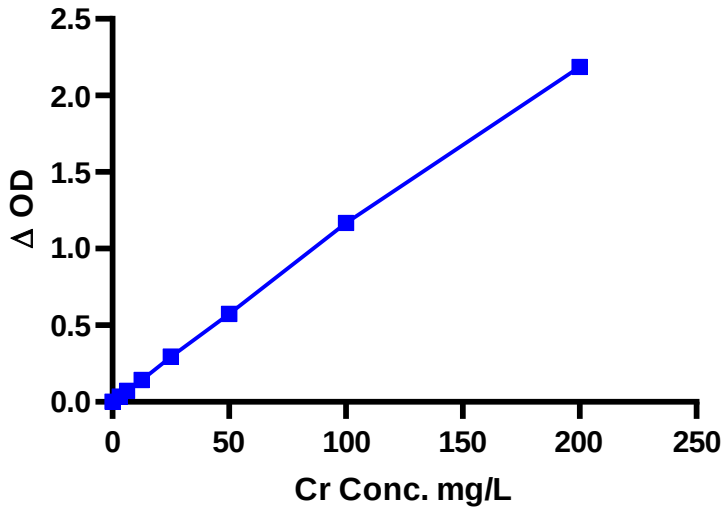
Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs for the zero standard from all OD values
  (for example if the OD value of zero standard, and standard 6 are 0.087, and 1.086 respectively; then the adjusted ODs equal 0 and 0.999 respectively.)
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 1 mg/L Creatinine is equivalent to 8.84 µM Creatinine



A typical standard curve of ZellX® UCr Assay kit

Run your own standard curves for calculation of results

Assay range

The limit of detection of ZellX® UCr assay was determined as 0.37 mg/L.

Sensitivity

The sensitivity of the ZellX® UCr assay was determined as 0.19 mg/L.

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Precision

Intra-Assay Precision (Precision within an assay): 4 human urine samples were tested 20 times in an assay.

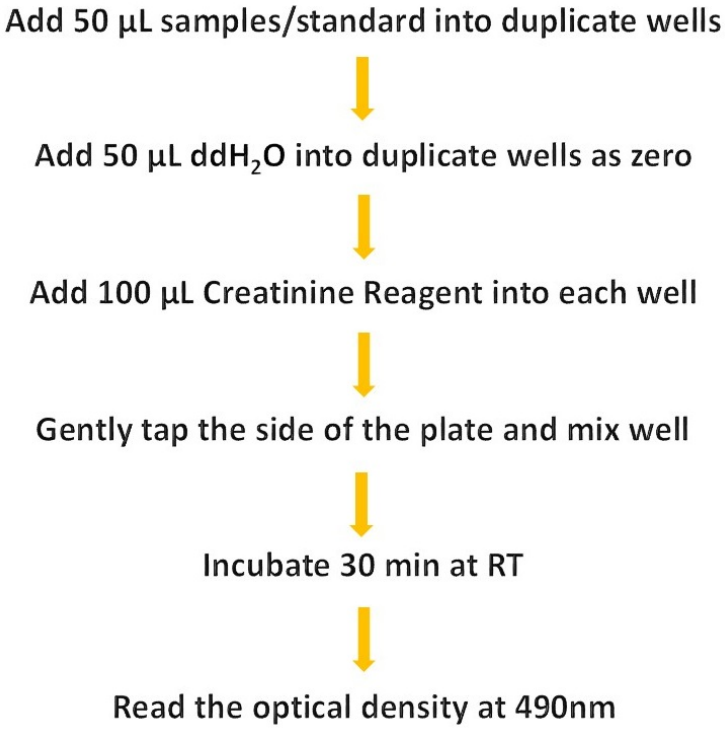
Inter-Assay Precision (Precision between assays): 4 human urine samples were tested in duplicate on 20 different assays over multiple days.

Item	*%CV*
Intra assay	2.5, 2.8, 3.0, 1.3
Inter assay	2.7, 3.7, 2.3, 3.9

Interferences

It is well known that some typical components of human urine may interfere with the Jaffe reaction for creatinine measurement in urine. A diluted urine sample was spiked with 20,000 mg/L of glucose (equivalent to 400,000 mg/L undiluted) and tested in the kit. The unspiked diluted sample read at 84.4 mg/L. No significant change to the measured creatinine level was seen at any glucose concentration.

Protocol summary



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References

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