Serum Creatinine (SCr)

Colorimetric Assay kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44111-96

www.zellx.de

Sample Types Validated for:

Human, Mouse, Rabbit, Rat and Sheep serum and EDTA and heparin plasma samples

!!! 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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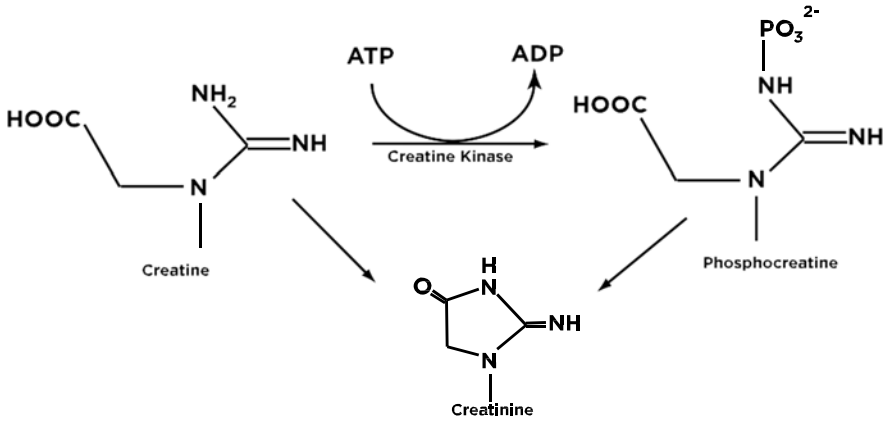
Introduction

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® serum Creatinine Kit is designed to quantitatively measure creatinine present in serum 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 assay utilizes a kinetic absorbance method to overcome interference by colored compounds in serum. The optical absorbance of the colored product is read after 1 minute in a microtiter plate reader capable of measuring 490 nm wavelength. At 30 minutes the optical density (OD) is read again. The concentration of creatinine is calculated using the delta of the OD readings at 30 and 1 minute compared to the curve generated from the standards. The Jaffe reaction used in this kit has been modified to read creatinine levels in serum.

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General information

Materials supplied in the Kit

Component	*Quantity*
Creatinine Standard (1000 mg/L)	50 µL
Creatinine Reagent	10 mL
Assay Diluent	2.6 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.

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- The instruction must be strictly followed. The reading of Microplate/ELISA reader must be set at the appropriate wavelength of determining the experiment result.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Assay protocol

Sample preparation

All samples should be centrifuged for 15 minutes at 14,000 rpm in an Eppendorf type centrifuge prior to conducting assay**.**

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

All samples must be used within 2 hours of dilution.

Standard preparation

- Prepare a 1:25 dilution of Creatinine Standard with ddH_2O (mix 10 µL of standard with 240 µL of ddH_2O), and label as the Standard No.4 (40 mg/L).
- Make series of lower dilutions as described in the table.
- ddH_2O is used as the 0 mg/L standard.

No.	*Concentration*	*Material needed*
Standard No.4	40 mg/L	10 µL Creatinine Standard + 240 µL ddH_2O
Standard No.3	20 mg/L	100 µL Standard No.4 + 100 µL ddH_2O
Standard No.2	10 mg/L	100 µL Standard No.3 + 100 µL ddH_2O
Standard No.1	5 mg/L	100 µL Standard No.2 + 100 µL ddH_2O
Standard No.0	0 mg/L	100 µL ddH_2O

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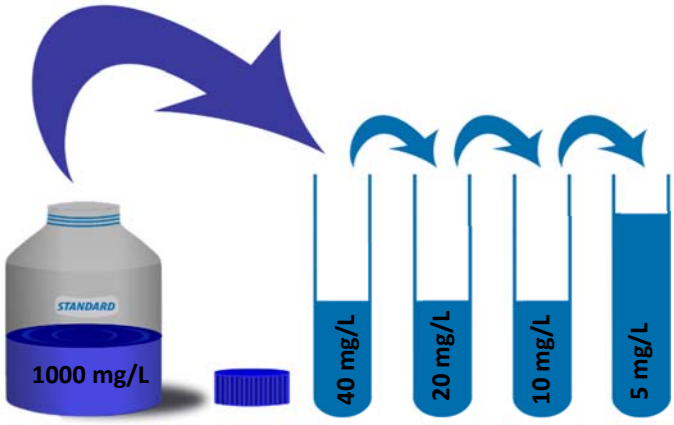

All standard must be used within 2 hours of preparation

Assay Procedure

1. Pipet 25 µL of either samples or standards into duplicate wells in the plate.
2. Pipet 25 µL of ddH_2O as the zero standard.
3. Add 25 µL of Assay Diluent to each well using a multichannel pipet. Remove the bobbles prior to addition of Reagent.
4. Add 100 µL of Creatinine Reagent to each well using a multichannel pipet.
5. Incubate at room temperature (RT).
6. Read the OD at 490 nm in 1 and 30 minutes after adding Creatinine Reagent.

Calculation

- Average the duplicate OD readings for each standard and sample.
- Subtract the average OD of the standards at 1 minute from the average OD of the standards at 30 minutes and plot the result (Average Delta OD) versus the creatinine concentration of the standards.
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted ΔOD values.

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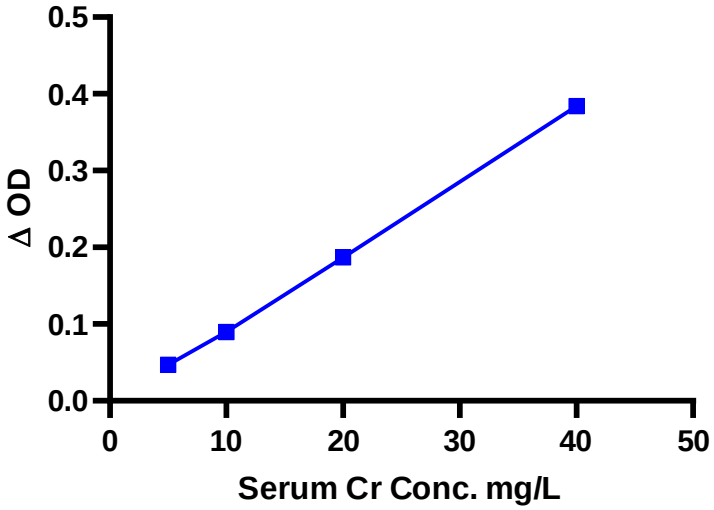
- Generate a linear regression line and use the equation, y=mx+b (y=Average delta OD; x=Creatinine Concentration: m=slope and b=intercept) to calculate the concentrations in the unknown samples.
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 10 mg/L Creatinine is equivalent to 88.40 µM Creatinine



A typical standard curve of ZellX® SCr Assay kit

Run your own standard curves for calculation of results

Sensitivity

The sensitivity of the ZellX® SCr assay was determined as 0.81 mg/L.

Precision

Intra-Assay Precision (Precision within an assay): 3 human serum samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 human serum samples were tested in duplicate on 19 different assays over multiple days.

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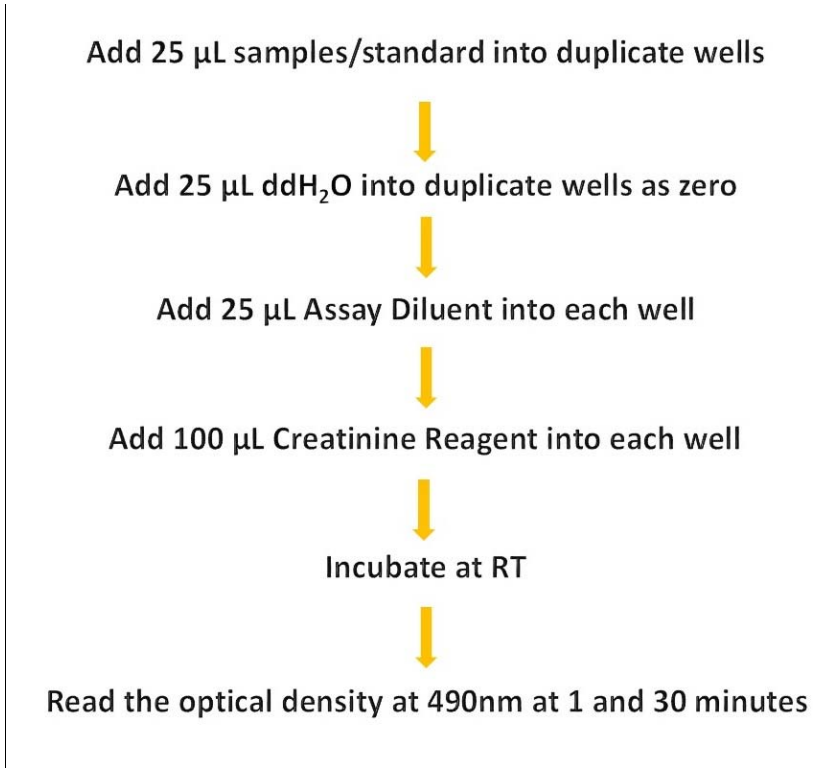
Item	*%CV*
Intra assay	4.5, 6.3, 7.9
Inter assay	8.0, 9.6, 7.3

Protocol summary



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