StressMarq Biosciences INC.

Discovery through partnership | Excellence through quality

StressXpress®

Creatinine Serum Detection Kit

Catalog# SKT-217 (2 Plate Kit)

Colorimetric measurement of creatinine

EAGLE BIOSCIENCES
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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-217A	Clear 96 well Plates	2 plates	Bag containing 2 by 96 well Half-Area plates.
SKC-217B	Creatinine Standard (Calibrated to NIST Standard Reference Material Lot Number 914a)	100 µL	A 100 mg/dL creatinine solution in deionized water.
SKC-217C	Assay Diluent	6 mL	A special diluent for use in the serum kit.
SKC-217D	StressXpress® Creatinine Reagent	20 mL	-

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

WARNING: Not for human or animal disease diagnosis or therapeutic drug use.

Precautions

As with all such products, 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 solution should not come in contact with skin or eyes. 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.

Storage Instructions

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

Materials Needed But Not Supplied

- Distilled or deionized water.
- Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25 and 100 µL.
- Colorimetric 96 well microplate reader capable of reading optical density at 490 nm.
- Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Please read this insert completely prior to using the product.

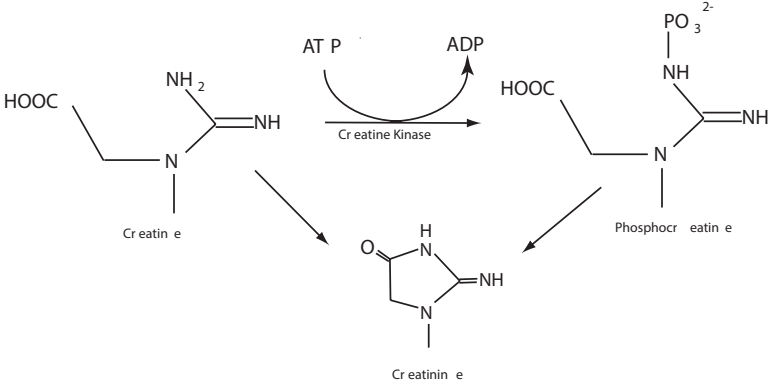
FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Creatinine (2-amino-1-methyl-5H-imadazol-4-one) is a metabolite of phosphocreatine (p-creatine), a molecule used as a store for high-energy phosphate that can be utilized by tissues for the production of ATP (1). Creatine either comes from the diet or synthesized from the amino acids arginine, glycine, and methionine. This occurs in the kidneys and liver, although other organ systems may be involved and species-specific differences may exist (2). Creatine and p-creatine are converted non-enzymatically to the metabolite creatinine, which diffuses into the blood and is excreted by the kidneys. *In vivo*, this conversion appears to be irreversible and in vitro it is favored by higher temperatures and lower pH2. Creatinine forms spontaneously from p-creatine (3). Under normal conditions, its formation occurs at a rate that is relatively constant and as intra-individual variation is <15% from day to day, creatinine is a useful tool for normalizing the levels of other molecules found in urine. Additionally altered creatinine levels may be associated with other conditions that result in decreased renal blood flow such as diabetes and cardiovascular disease (4-6).



Assay Overview

The StressXpress® Creatinine Serum Kit is designed to quantitatively measure creatinine present in serum samples. Please read the complete kit insert before performing this assay. A creatinine standard, calibrated to a NIST creatinine standard, is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or 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 StressXpress® Creatinine Reagent, which is pipetted into each well.

The assay utilizes a kinetic absorbance method to overcome interference by colored compounds in serum. The absorbance of the colored product is read after 1 minute in a microtiter plate reader capable of measuring 490nm wavelength. At 30 minutes the optical density is read again. The concentration of creatinine is calculated using the delta of the optical density readings at 30 and 1 minute compared to the curve generated from the standards, or by using the Excel worksheet available for free download at our web site. The Jaffe reaction used in this kit has been modified to read creatinine levels in serum,8.

PRE-ASSAY PREPARATION

Sample Types

Sample Types Validated:
Mammalian Serum and Plasma

This assay has been validated for human, mouse, rabbit, rat and sheep serum and EDTA and heparin plasma samples. The end user should evaluate recoveries of creatinine in other plasma and serum samples being used.

For measuring Creatinine in urine samples, please refer to our StressXpress® Creatinine Urinary Detection kits, Catalog # SKT-200.

Hemolyzed or lipemic samples should not be used with this kit. Hemolyzed samples have shown a decrease in creatinine concentration with increasing hemoglobin, whereas lipemic samples have been shown to yield artificially high creatinine concentrations.

Sample Preparation

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

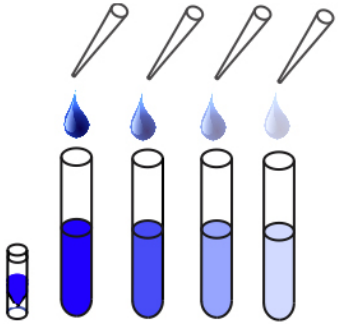
Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine creatinine concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Standard Preparation

Label four glass test tubes #1 through #4. Pipet 240 µL of water into tube #1 and 100 µL into tubes #2-#4. Carefully add 10 µL of the Creatinine stock solution to tube #1 and vortex completely. Take 100 µL of the creatinine solution in tube #1 and add it to tube #2 and vortex completely. Repeat these serial dilutions for tubes #3 and #4. The concentration of creatinine in tubes 1 through 4 will be 4, 2, 1 and 0.5 mg/dL. Water is used as a sample blank of 0 mg/dL.

Use all Standards within 2 hours of preparation.



	Standard 1	Standard 2	Standard 3	Standard 4
Water Volume (µL)	240	100	100	100
Addition	Stock	Standard 1	Standard 2	Standard 3
Volume of Addition (µL)	10	100	100	100
Final Concentration (mg/dL)	4	2	1	0.5

Performing the Assay

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.

2 Pipet 25 µL of samples, water as the blank, or standards into wells in the clear plate.

3. Add 25 µL of Assay Diluent to all wells used. Allow to warm completely to Room Temperature prior to use. Set a timer to read 30 minutes and ensure that the plate reader is set to read optical density at 490 nm.

4. Observe wells, checking for bubbles. If bubbles are present, tap the plate gently to remove prior to addition of Reagent.

5. Add 100 µL of the StressXpress® Creatinine Reagent to each well using a repeater pipet. Immediately start the timer after adding the Creatinine Reagent to the last well.

6. Incubate at room temperature.

7. At 1 minute, read the optical density generated from each well in a plate reader capable of reading at 490 nm.

8. At 30 minutes, again read the 490 nm optical density generated from each well in the plate reader.

ANALYSIS

Calculation of Results

Subtract the average Optical Density of the standards at 1 minute from the average Optical Density of the standards at 30 minutes and plot the result (Average Delta OD) versus the creatinine concentration of the standards. 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.

Typical Data

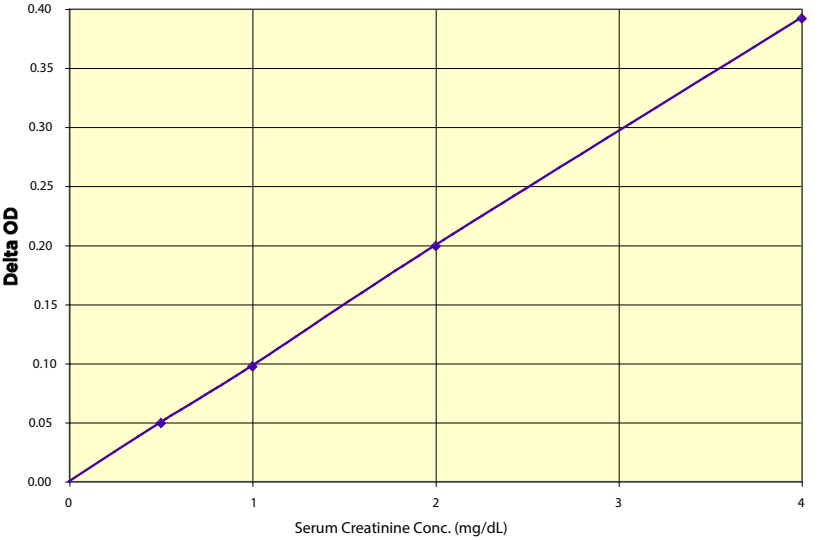
Sample	Net Delta OD	Creatinine Concentration (mg/dL)
Standard 1	0.393	4
Standard 2	0.201	2
Standard 3	0.099	1
Standard 4	0.051	0.5
Sample 1	0.134	1.35
Sample 2	0.107	1.08

Always run your own standard curve for calculation of results.
Do not use this data.

Creatinine standard calibrated to NIST Standard Reference Material Lot Number 914a

Conversion Factor: 1 mg/dL Creatinine is equivalent to 88.40 µM Creatinine

Typical Standard Curve



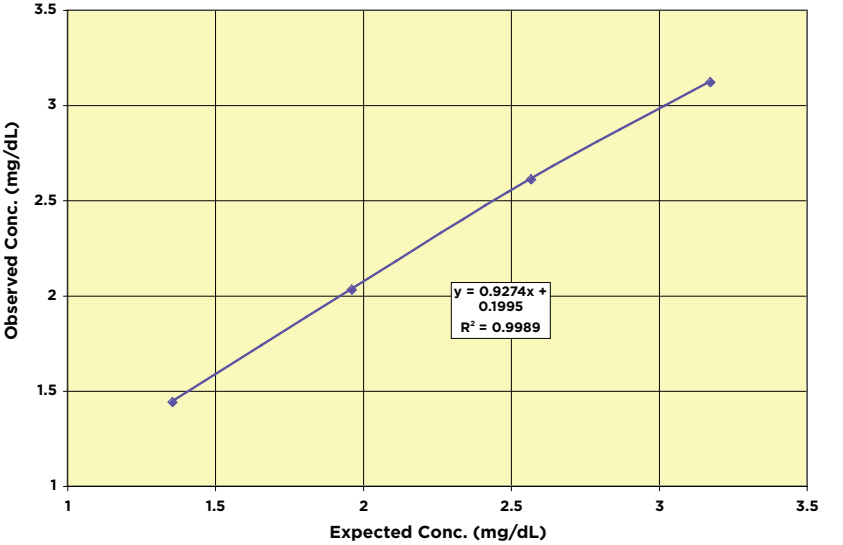
Always run your own standard curves for calculation of results.
Do not use this data.

Validation Data

Linearity

Linearity was determined by taking two human serum samples, one with a low diluted creatinine level of 0.75 mg/dL and one with a higher level of 3.78 mg/dL and mixing them in ratios given below. The measured concentrations were compared to the expected values.

Low Serum	High Serum	Observed Concentration (mg/dL)	Expected Concentration (mg/dL)	% Recovery
80%	20%	1.44	1.36	106.2%
60%	40%	2.03	1.96	103.5%
40%	60%	2.61	2.57	101.6%
20%	80%	3.12	3.17	98.3%
			Mean Recovery	102.4%


3.5
3
2.5
2
1.5
1
Observed Conc. (mg/dL)
y = 0.9274x + 0.1995
R² = 0.9989
1
1.5
2
2.5
3
3.5
Expected Conc. (mg/dL)

Intra Assay Precision

Three human serum samples were run in replicates of 20 in an assay. The mean and precision of the calculated creatinine concentrations were:

Sample	Creatinine Concentration (mg/dL)	%CV
1	0.99	7.9
2	1.50	6.3
3	3.82	4.5

Inter Assay Precision

Three human serum samples were run in duplicates in 19 assays run over two years by four operators. The mean and precision of the calculated creatinine concentrations were:

Sample	Creatinine Concentration (mg/dL)	%CV
1	0.91	9.6
2	1.26	7.3
3	3.51	8.0

Sample Values

Eleven serum samples from a variety of different species were tested in the assay. Values ranged from 0.78 to 1.45 mg/dL with an average of 1.00 mg/dL.

Cross Reactivity

It is well known that some typical components of serum may interfere with the Jaffe reaction for creatinine measurement (7,8).

A serum sample was spiked with varying concentrations of bilirubin and tested in the assay. Bilirubin level in normal serum is between 0.2 and 1.0 mg/dL (9). The unspiked sample read at 0.86 mg/dL. No significant change to the measured creatinine level was seen up to an additional 1.0 mg/dL of bilirubin.

References

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Warranty and Limitation of Remedy

StressMarq Biosciences Inc. makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. StressMarq **warrants only** to the original customer that the material will meet our specifications at the time of delivery. StressMarq will carry out its delivery obligations with due care and skill. Thus, in no event will StressMarq have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if StressMarq is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of StressMarq, its directors or its employees.

Buyer's **exclusive remedy** and StressMarq's sole liability hereunder shall be limited to a refund of the purchase price, or at StressMarq's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to StressMarq within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Refund Policy located on our website and in our catalog.

Contact Information

Technical Service Contact Information

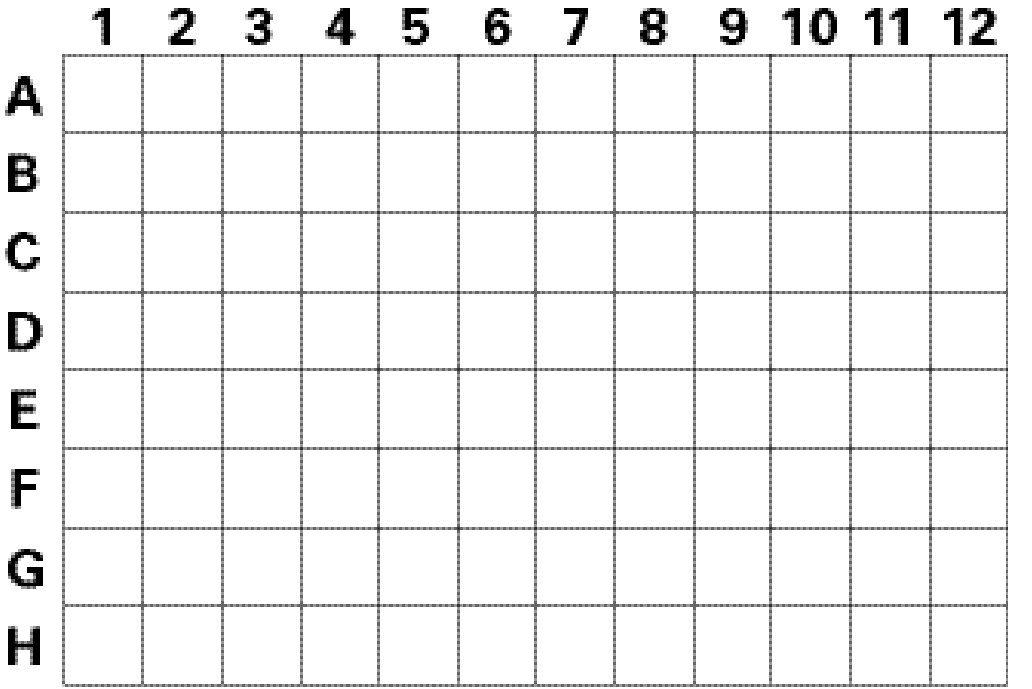
Phone: 250-294-9065

Fax: 250-294-9025

E-Mail: techsupport@stressmarq.com

Hours: M-F 9:00 AM to 5:00 PM PST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).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