ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
N-CAL™ KIT

NCal™ International Standard Kit

DetectX®

Serum Creatinine
Low Sample Volume Detection Kit

384-Well Plate Kit Catalog Number KB02-H1D

Species Independent

Sample Types Validated:
Mammalian Serum and Plasma

Calibrated to NIST Standard Reference Material Lot No. 914a

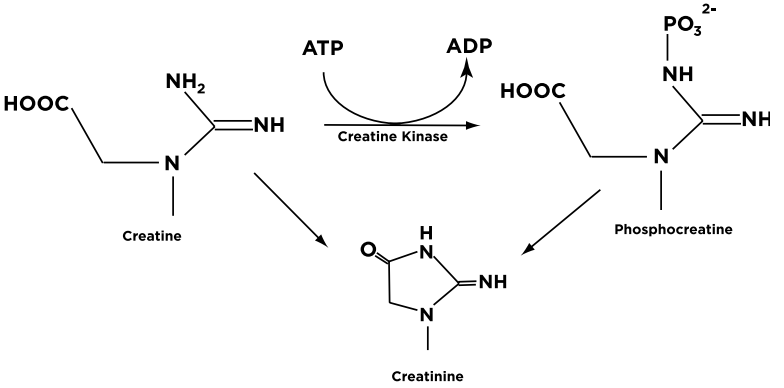
Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

TABLE OF CONTENTS

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 pH[2]. 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].



1. Wallimann, T. et al., Biochem. J., 2000, 281, 21-40.
2. Wyss, M. and Kaddurah-Daouk, R., Physiol. Rev., 2000, 80, 1107-1213.
3. Raja Iyengar, M. et al., J. Biol. Chem, 1985, 260, 7562-7567.
4. Manjunath, G. et al., Postgrad. Med. 2001, 110, 55-62.
5. Gross, J.L. et al., Diabetes Care, 2005, 28, 164-176.
6. Anavekar, N.S. et al., New Engl. J. Med., 2004, 351, 1285-1295.

ASSAY PRINCIPLE

The DetectX® Low Sample Volume Serum Creatinine Kit is designed to quantitatively measure creatinine present in serum samples using a 384-well plate format to minimize sample usage. Only 15 µL of serum or plasma is needed per well. 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 the clear microtiter plate. An assay diluent is added to all standards, controls and samples. The color generating reaction is initiated with the DetectX® 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].

RELATED PRODUCTS

Kits	Catalog No.
Hemoglobin High Sensitivity Colorimetric Detection Kits	K013-HX1/HX5
Human Cystatin C ELISA Kit	K012-H1
RBP Multi-Format ELISA Kits	K062-H1/H5
Urinary Creatinine Detection Kits	K002-H1/H5

SUPPLIED COMPONENTS

Clear 384 well Plate

Bag containing a 384-well plate. See: www.ArborAssays.com/resources/#general-info for plate dimension data.

Kit KB02-H1D	1 Each	Catalog Number X105-1EA

Creatinine Standard

A 100 mg/dL creatinine solution in deionized water. *Calibrated to NIST Standard Reference Material Lot Number 914a.*

100 µL	Catalog Number C003-100UL

Assay Diluent

A special diluent for use in the serum kit. Allow to warm completely to **Room Temperature** prior to use.

12 mL	Catalog Number X017-12ML

DetectX® Creatinine Reagent

28 mL	Catalog Number C004-28ML

STORAGE INSTRUCTIONS

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

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Appropriate manual or automated dispensing equipment for adding 15 or 60 µL of reagents to 384-well plates.

If you are not using an automated system then a repeater pipet for dispensing 60 µL of reagent is needed with disposable tips.

Colorimetric reader capable of reading 384-well microplate 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.

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.

SAMPLE TYPES

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 DetectX® Urinary Creatinine Detection kits, Catalog Number K002-H1 or K002-H5.

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. Please see our Hemoglobin Detection kit, K013-H1 for details of a convenient method to measure Hb levels in whole blood and Hemoglobin High Sensitivity Detection Kits, K013-HX1/HX5, for serum and plasma.

SAMPLE PREPARATION

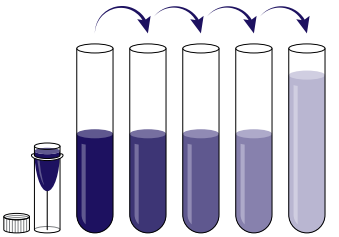
All samples should be centrifuged for 15 minutes at 14,000 rpm in an Eppendorf type centrifuge prior to running in the assay.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Standard Preparation

Label glass test tubes #1 through #4. Pipet 120 µL of water into tube #1 and 50 µL into tubes #2-#4. Carefully add 5 µL of the Creatinine stock solution to tube #1 and vortex completely. Take 50 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.

	Std 1	Std 2	Std 3	Std 4
Water Volume (µL)	**120**	50	50	50
Addition	Stock	Std 1	Std 2	Std 3
Volume of Addition (µL)	**5**	50	50	50
Final Conc (mg/dL)	4	2	1	0.5

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine creatinine concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.
2. Pipet 15 µL of samples, water as the blank, or standards into wells in the clear plate.
3. Add 15 µL of Assay Diluent to all wells used after allowing to warm completely to **Room Temperature**. 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 60 µL of the DetectX® Creatinine Reagent to each well using a repeater pipet or other pipetting equipment. 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.

NOTE: *If you do not use the whole plate, mark the wells that have been used. The unused wells can be used for further measurements.* ***Do not wash wells to reuse.***

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.

Alternatively go to our website and download a sample concentration spreadsheet at:
www.ArborAssays.com/resources

Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-creatinine-serum-kit.assay

*The MyAssays logo is a registered trademark of MyAssays Ltd.

TYPICAL DATA

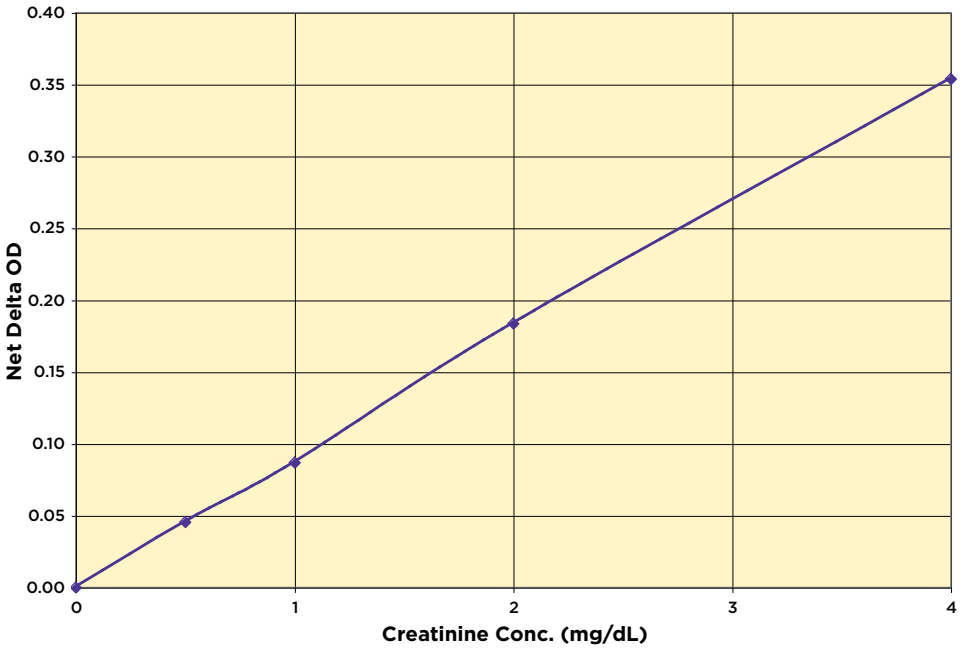
Sample	Net Delta OD	Creatinine Conc. (mg/dL)
Standard 1	0.354	4
Standard 2	0.184	2
Standard 3	0.087	1
Standard 4	0.046	0.5
Sample 1	0.100	1.11
Sample 2	0.069	0.76

Data shown above was generated by Chris Cheung from Andrea Graham's lab, the Department of Ecology and Evolutionary Biology, Princeton University. Website: www.princeton.edu/~algraham

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.

Data shown above was generated by Chris Cheung from Andrea Graham's lab, the Department of Ecology and Evolutionary Biology, Princeton University. Website: www.princeton.edu/~algraham

VALIDATION DATA (FROM 96-WELL KIT)

Sensitivity

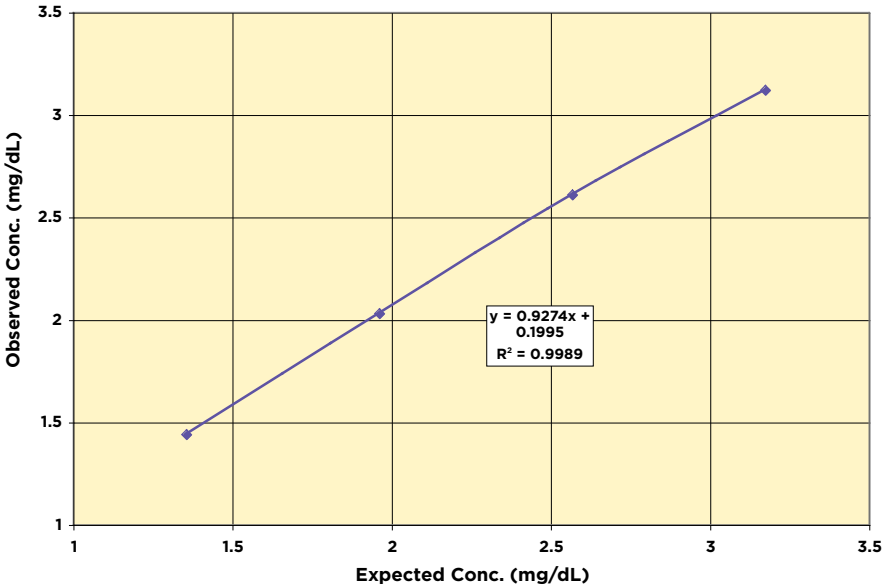
Sensitivity was calculated by comparing the Delta ODs obtained for twenty wells run for each of the zero and standard #4. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.081 mg/dL.**

VALIDATION DATA (FROM 96-WELL KIT)

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	Expected Conc. (mg/dL)	Observed Conc. (mg/dL)	% Recovery
80%	20%	1.36	1.44	106.2%
60%	40%	1.96	2.03	103.5%
40%	60%	2.57	2.61	101.6%
20%	80%	3.17	3.12	98.3%
			Mean Recovery	**102.4%**



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 Conc. (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 Conc. (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 AND INTERFERENTS

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.

7. Cook, J.G.H., Ann Clin. Biochem., 1975, 12, 219-232.
8. Young, D.D., in “Effects of Drugs on Clinical laboratory Tests”, 1990.
9. Tietz, N.W., Textbook of Clinical Chemistry, WB Saunders, 1986.

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

FSC Printed on Forest Stewardship Council certified paper